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# BULLETIN

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# Studies on Experimental Hypophysectomy

## I. Effect on the Maintenance of Life

By Walter E. Dandy, M.D., and Frederick Leet Reichert, M.D.

*From the Department of Surgery, The Johns Hopkins University*

STUDIES following extirpation of the hypophysis in animals have led to confusion rather than to clearness. One group of investigators arrives at the conclusion that most of the somatic characteristics, normal and abnormal, arise from this structure—even that life depends upon it—whereas another group looks upon it as quite useless or at most unimportant. And strangely enough, these widely divergent results are attained from experiments which, to outward appearances at least, seem very similar. We are, therefore, left with the impression that there must be not only the important element of personal equation in overdrawing conclusions, but that in the performance of the experiments there must be flaws which have remained hidden.

It is the purpose of this communication to present experimental evidence upon only one phase of alleged pituitary function; namely, Is the pituitary essential to life? We now believe it is easy not only to explain the contradictory results, but also to correlate conclusions which are so far apart.

In view of the fact that the literature on hypophyseal experiments has been so fully and frequently covered, we shall only briefly refer to those reports which bear particularly upon the relationship of the pituitary to the maintenance of life. Victor Horsley, in 1886,[1] published a brief note on the removal of the hypophysis in dogs, using a lateral temporal approach. Two of the animals lived five and six months and showed nothing that distinguished them from other dogs. Unfortunately, no gross or microscopic necropsy reports were made. For twenty years these results remained unquestioned. In 1908, Gemelli[2] removed the hypophysis of cats, using a buccal route, and obtained results identical with those of Horsley.

Paulesco (1908),[3] slightly altering the operation by opening a vent in the bone and dura of the contralateral side of the skull, arrived at the sensational conclusion that the hypophysis was necessary to life. Out of twenty-two dogs with total extirpations, not one survived longer than forty-eight hours, and all except two died in less than thirty-six hours. From a more

[1] Horsley (Victor): Functional nervous disorders due to loss of thyroid gland and pituitary body. Abstract of third Brown Lecture. *Lancet*, 1886, i, 5.

[2] Gemelli: Sur la fonction de l'hypophyse. *Arch. ital. de biol.*, 1908, L, 157–174.

[3] Paulesco (N. C.): L'Hypophyse du Cerveau. Vigot Frères, Eds., Paris, 1908.



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extended series there were, however, several recoveries which he explained by only "partial" removal of the gland. The object of the second cranial opening was to afford, by dislocation of the brain, a better exposure of the hypophyseal region.

Cushing[4] and his coworkers—Reford, Crowe and Homans—using essentially the same bilateral approach, confirmed Paulesco's findings in every respect. Their experiments have been controlled by gross and microscopic studies of the base of the brain after the death of the animals. The protocols by Crowe, Cushing and Homans[5] have been presented in great detail and with such accuracy that they still offer an unusual opportunity for study and probably different interpretation even at this time.

Stimulated by the above experiments, Handelsmann and Horsley[6] removed the hypophysis from a larger series of dogs, controlled the extirpations by post-mortem microscopic studies of the base of the brain, and again arrived at precisely the same conclusions which Horsley had originally published, namely, that the hypophysis gave no evidence of any important function.

Since then numerous authors have published results some of which are identical with those of Horsley—Aschner[7] (1912), Sweet and Allen (1913)[8] Benedict and Homans (1912),[9] Camus and Roussy (1913),[10] and Brown (1923).[11] Others, however, have agreed with Paulesco—Reford and Cushing, Crowe, Cushing and Homans, Houssay (1916),[12] Bell (1917),[13] Dott (1923).[14]

That the pituitary is not essential to the life of tadpoles, has been conclusively demonstrated by the extirpation experiments of Smith (1916)[15] and of Allen (1916),[16] and more

[4] Reford (L. L.) and Cushing (H.): Is the pituitary gland essential to the maintenance of life? *Johns Hopkins Hosp. Bull.*, 1909, xx, 105.

[5] Crowe (S. J.), Cushing (H.) and Homans (J.): Experimental hypophysectomy. *Johns Hopkins Hosp. Bull.*, 1910, xxi, 127.

[6] Handelsmann and Horsley (V.): Preliminary note on experimental investigations on the pituitary body. *Brit. Med. Jour.*, 1911, ii, 1150.

[7] Aschner (B.): Ueber die Funktion der Hypophysis. *Arch. f. d. ges. Physiol.*, 1912, cxlvi, 1.

[8] Sweet (J. E.) and Allen (A. R.): The effect of the removal of the hypophysis in the dog. *Ann. Surg.*, 1913, lvii, 485.

[9] Benedict (F. G.) and Homans (J.): The metabolism of the hypophysectomized dog. *Jour. Med. Research*, 1912, xx, 409.

[10] Camus (J.) and Roussy (M. G.): Présentation de sept chiens hypophysectomisés depuis quelques mois. *Soc. de biol.*, 1913, lxxiv, 1386.

[11] Brown (C. G.): The effects of complete extirpation of the hyphysis in the dog (preliminary report). *Proc. Soc. Exper. Biol. and Med.*, 1922-23, xx, 275.

[12] Houssay (B. A.): Extirpación de la hypófisis en el perro. First Natl. Cong. Med., Buenos Aires, 1916. Abstr. in *Endocrin.*, 1918, ii, 497.

[13] Bell (W. B.): The Pituitary. Baillière, Tindall & Cox, London, 1919. *Also: Quart. Jour. Exper. Physiol.*, 1917, xi, 78.

[14] Dott (N. M.): An investigation into the function of the pituitary and thyroid glands. Part I. The technique of their experimental surgery and summary of results. *Quart. Jour. Exper. Physiol.*, 1923, xiii, 241.

[15] Smith (P. E.): Experimental ablation of the hypophysis in the frog embryo. *Science, N. S.*, Aug. 25, 1916, p. 280. *Also: Anat. Rec.*, 1916, xi, 57.

[16] Allen (B. M.): Extirpation experiments in Rana pipiens larvae. *Science, N. S.*, 1916, xliv, 1143.

recently of others. They removed the pharyngeal bud of the hypophysis from very young frog embryos. For the time being we are passing over, as irrelevant to our subject, the profound disturbances of growth and pigmentation which have followed extirpation of this hypophyseal anlage. These results have been consistent in the hands of numerous investigators; they have not been and are not challenged.

physis has offered great difficulties of operative approach preparatory to its extirpation. Two methods have been evolved to reach this pea-sized structure: (1) the temporal or intracranial, and (2) the buccal. The former approach used by Horsley (unilateral), Paulesco, Cushing, Sweet and Allen, Bell, Dott, Houssay, Benedict and Homans (bilateral), has the advantage of being an aseptic route. It is particularly useful in dogs because the sella turcica is shallow and a satisfactory exposure of the hypophysis can be obtained from the side. This method, however, is impracticable in cats, for the sella is deeper and the curved and overhanging posterior clinoid process envelops the posterior half of the hypophysis and hides it from view. The buccal route is also through a septic field, and its use has been

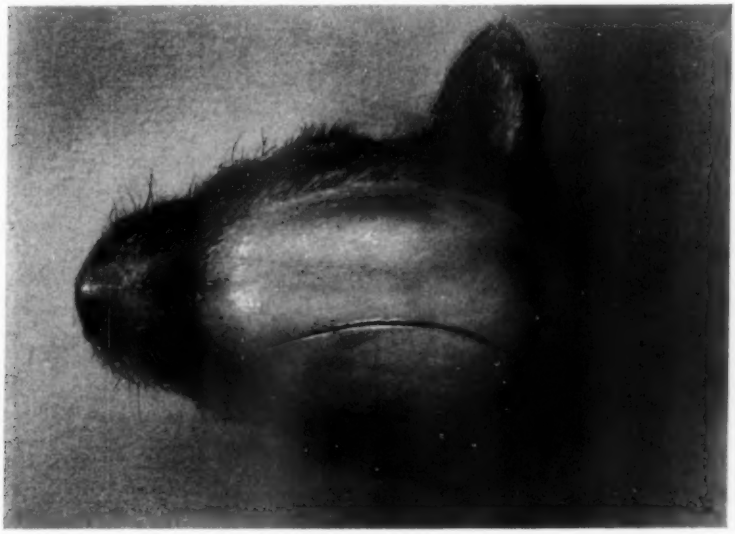

FIG. 1. THE CURVED SKIN INCISION CORRESPONDS WITH THE INCISION THROUGH THE TEMPORAL MUSCLE BENEATH

So far as the alleged relation of the hypophysis to life is concerned, our experiments carry precisely the same conclusion for mammals (dogs) which their experiments have demonstrated for amphibia (frogs)—*i.e.*, that the hypophysis is not concerned with the maintenance of life.

Well secluded at the base of the brain and skull and attached to both almost at the central point, the hypo-

attended by a considerable mortality from meningitis and localized infections. It is, however, the only feasible approach for the extirpation of the feline hypophysis. This method was employed in dogs or cats by Gemelli, Aschner, Camus and Roussy, and Brown.

maze of contradictory results and interpretations, one lead is striking and probably significant, namely, that of the above mentioned investigators all who have used the buccal route are in agreement that the pituitary body is not essential to life. The only disagreement, therefore, is among those

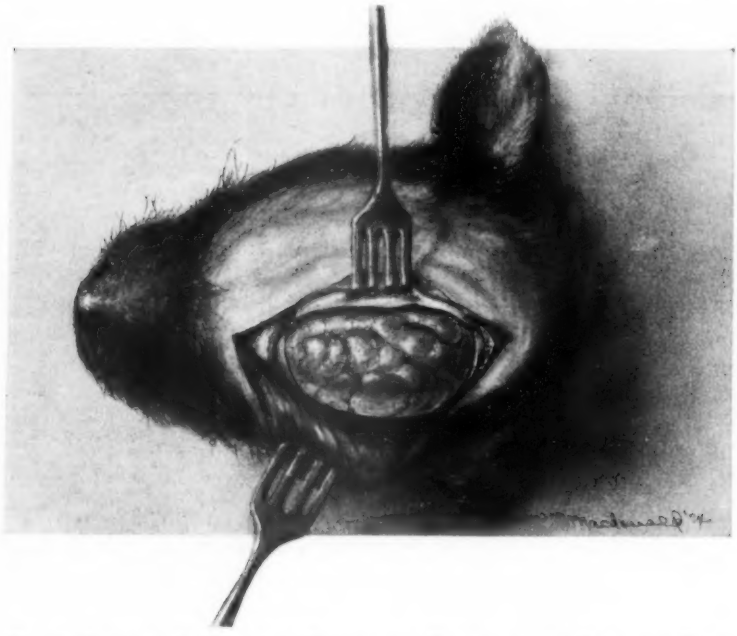

FIG. 2 SHOWING APPROXIMATE SIZE OF THE DEFECT IN THE BONE AND DURA

Those who support the view that the hypophysis is essential to life believe that microscopic fragments have been left in the surviving animals and they demand microscopic proof that anterior lobe cells are absent. But Horsley and Handlesmann, Aschner, Gemelli, and Sweet and Allen, have controlled their experiments microscopically to meet this objection.

In attempting an analysis of this who have employed the temporal (intracranial) route. That the type of operation has much to do with the divergent results, will be shown later.

For several years we have been experimenting with the intracranial approach to the canine hypophysis. At first some of our dogs died just as did those of Paulesco, Cushing, and others, but some lived (adults) and seemed little if any worse for the

operation, either immediately or subsequently. Although, to all appearance, precisely the same procedure was used and as far as we could judge the extirpation was done with equal care and thoroughness, we were confronted with totally different results. It was at once evident that the life of the animal was not dependent upon "microscopic remnants of the pituitary," for many of our animals were subjected to careful microscopic control. It was also clear that if a few animals survived a total hypophysectomy, there must be an explanation other than loss of "hypophyseal function" for the death of the remainder. The preservation of life in one totally hypophysectomized animal is sufficient denial of the theory that the hypophysis is essential to life.

There seemed but one possible reason for these seemingly paradoxical results, namely, that in some way the operative procedure itself must be held accountable. The fact that death following hypophysectomy usually occurs in less than forty-eight hours (at times after a longer period), is in itself sufficient reason to place the burden of proof on the operator. Moreover, the lassitude, drowsiness,

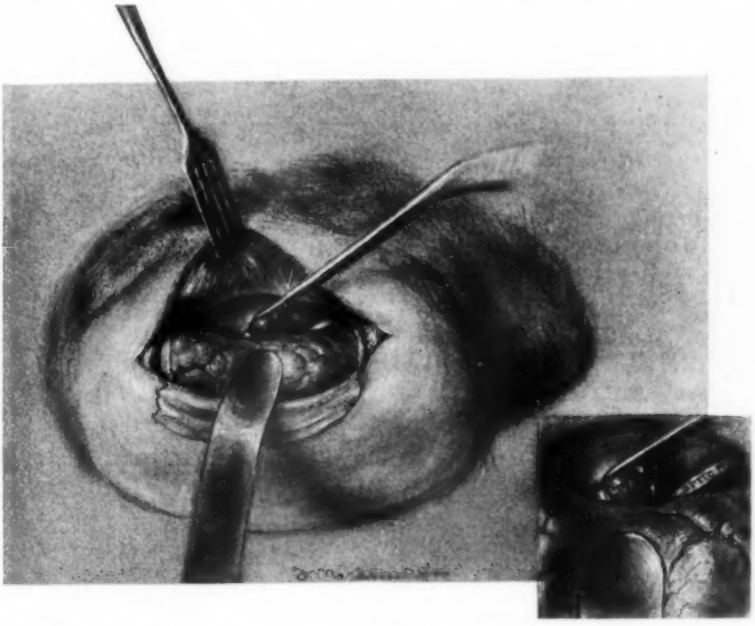

FIG. 3. THE HEAD IS NOW INVERTED SO THAT THE BRAIN MAY HANG AWAY FROM THE BASE OF THE SKULL, THUS REDUCING TO A MINIMUM THE TRACTION WITH THE SPATULA

The forceps are shown separating the pituitary from its attachment to the posterior clinoid process. The carotid artery is just anterior to the hypophysis and behind the optic nerve. The cut stump of the oculomotor nerve is shown just posterior to the hypophysis.

and coma—all not infrequently appearing after a quick recovery from the anaesthetic—the not infrequent convulsions, were precisely like the manifestations (complications) which follow human intracranial operations. They are evidences of pressure

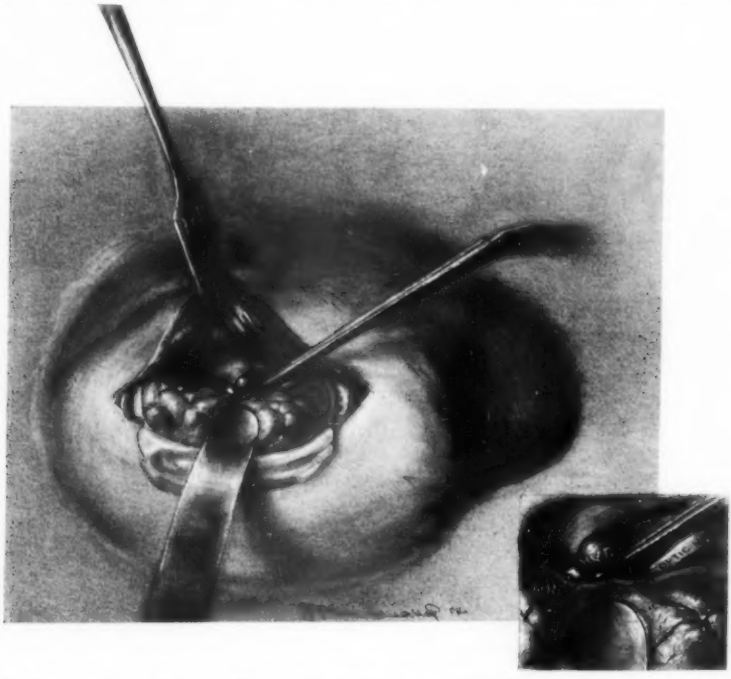

FIG. 4. SHOWING THE FORCEPS STRIPPING AND PINCHING THE STALK AT THE JUNCTION WITH THE BASE OF THE BRAIN

In the adult this exposure can be obtained by release of the fluid from the cisterna; in young puppies it can be obtained only by shrinking the brain with Weed's intravenous hypertonic salt solution.

(drowsiness and coma) and of local injury (convulsions).

Recently Bailey and Bremer,[17] working in Cushing's laboratory, obtained precisely the same symptoms—

[17] Bailey (P) and Bremer (F.): Experimental diabetes insipidus. *Arch. Int. Med.*, 1921, xxviii, 773.

lassitude, arched back, coma, convulsions, etc.—described by Cushing as characteristic of the loss of hypophyseal function—by puncture of the tuber cinereum. Though their experiments are mainly concerned with glycosuria and polyuria, the inference is left that the above symptoms are not of hypophyseal origin but are the result of injury to the tuber cinereum—an injury readily associated with hypophysectomy. It is clear that they are also dealing with cerebral trauma and the symptoms are those of intracranial pressure. Proof

of this is the entire absence of such symptoms in our experiments in which this entire region was cauterized.

### THE OPERATIVE PROCEDURE

Our efforts have been directed toward the development of a method as free from operative complications—trauma and haemorrhage—as possible. We have used only the temporal intracranial route as originally devised by Horsley and, like Horsley, have made only a unilateral bony opening. Almost from the outset it seemed to us that the contralateral subtemporal defect was of doubtful value in providing better exposure, and that the division of both temporal muscles might well be a factor in interfering with the intake of food after operation.

After dividing the temporal muscle parallel to its origin and stripping it from the skull, a large defect is made both of bone and dura (Figs. 1 and 2). Particularly important is the extension of the opening to the base of the skull. It is also advisable, though not necessary, to remove the zygoma, in order to give the lowest possible entrance to the base of the brain. While the convex inferior surface of the temporal lobe is slightly below a plane between the lower margin of the bony defect and the hypophysis, the spatula can be passed to the region of the sella without undue elevation of the lobe. An exposure of the hypophyseal region sufficient to permit painstaking removal of the pituitary body would, however, demand elevation and retraction of the temporal lobe—a trauma too severe and too prolonged. It is precisely this injury to the brain which has in large measure been responsible for the deaths (usually within forty-eight hours) reported by all investigators who have used this method of approach.

Trauma to the temporal lobe can be avoided or at least greatly reduced in two ways: (1) by inverting the head 100° to 135° so that the brain will fall away from the base of the skull; and (2) by releasing as much cerebrospinal fluid as possible.

After the dural opening has been made, the head is constantly maintained in the dependent position until the hypophysis has been removed (Fig. 3). Before attempting an exposure of the hypophyseal region, a small spatula is gently passed to the cisterna interpeduncularis, which is punctured. At once the release of fluid gives much additional room and with the hanging brain but little if any retraction with the spatula is necessary to afford an excellent view of the hypophyseal region and ample room for deliberate and careful removal of the hypophysis. The importance of these details, particularly the hanging brain, can be readily appreciated when it is realized that with the customary position of the head the entire brain must be lifted and held in position during the entire operation—in many of the reported procedures for one and a half to two hours. The effects of this long sustained cerebral trauma are not immediate, but appear some hours later. When sufficient room is obtained by the hanging brain, the spatula seems scarcely necessary except to protect the brain from the wisps of moist cotton which are used to sponge the hypophyseal region. The oculomotor nerve obliquely bisects the view of the

hypophysis and, since its function is of no concern, its division at the dural attachment is advisable. An unobstructed view of the hypophysis is then afforded.

The hypophysis is attached at only two points: (1) at the center of the posterior clinoid process where the posterior lobe artery enters, and (2) at the base of the brain by the stalk from which the hypophysis dangles below the cisterna chiasmatis and into

TABLE I

*Total hypophysectomy in adult dogs*

| ESTIMATED AGE | CAUTERIZATION OF BASE | POST-OPERATIVE | | | | | DURATION OF LIFE AFTER OPERATION | NECROPSY FINDINGS |
|---|---|---|---|---|---|---|---|---|
| | | Glycosuria | Polyuria | Signs of pressure | Convulsions | Motor weakness | | |
| 1 year | 0 | 0 | + | ++ | ++ | 0 | 24 days | Distemper. Some cerebral softening |
| 2 years | 0 | + | + | ++ | 0 | 0 | 81 days | Died in country |
| 1 year | 0 | 0 | 0 | 0 | ++ | + | 16 days | Distemper |
| 1–2 years | + | + | + | ++ | | | 1 year | Distemper. Emaciated |
| 3 years | + | + | 0 | + | ++ | 0 | 19 days | Distemper |
| 1 year | + | 0 | 0 | 0 | 0 | 0 | 24 days | Died in country |
| 1 year | + | + | 0 | 0 | 0 | 0 | 104 days | Emaciated |
| 2 years | + | 0 | 0 | + | ++ | + | 34 days | Died in country |
| 1 year | 0 | 0 | 0 | 0 | 0 | 0 | 75 days | Died in country |
| 2 years | 0 | + | 0 | 0 | 0 | 0 | 8 days | Distemper |
| 1–2 years | 0 | ++ | 0 | 0 | 0 | 0 | 144 days | Killed in fight |
| 1–2 years | 0 | + | 0 | 0 | 0 | 0 | 22 days | Distemper |
| 1 year | + | 0 | 0 | 0 | 0 | 0 | 69 days | Killed in fight |
| 2 years | ++ | 0 | 0 | 0 | 0 | 0 | 237 days | Poisoned in country |
| 7 months | ++ | 0 | + | 0 | + | 0 | 11 days | Cortical injury |
| 8 months | ++ | +++ | 0 | + | 0 | + | 138 days | Died in country |
| 7 months | + | 0 | 0 | 0 | 0 | 0 | 27 days | |
| 1 year | + | + | 0 | + | + | + | 1 year | Distemper |
| 2 years | ++ | +++ | + | + | | | 32 days | |
| 2 years | +++ | +++ | + | ++ | ++ | + | 2 days | Cortical softening; blood clot at base |
| 4 years | 0 | ++ | 0 | 0 | 0 | 0 | 138 days | |
| 1 year | 0 | | | | | | 2 days | Blood clot at base |
| 9 months | 0 | ++ | 0 | ++ | + | + | 5 days | Blood clot at base |
| 3 years | 0 | + | + | 0 | 0 | 0 | 90 days | Poisoned |
| 1 year | 0 | +++ | 0 | 0 | 0 | 0 | 11 days | Pneumonia |
| 2 years | 0 | 0 | 0 | 0 | 0 | 0 | 18 days | |
| 2 years | 0 | 0 | 0 | + | ++ | 0 | 12 days | Distemper |
| 1 year | 0 | + | 0 | ++ | + | 0 | 1 day | Blood clot at base |
| 2 years | 0 | 0 | + | 0 | 0 | 0 | 10 days | |
| 2 years | 0 | + | 0 | 0 | 0 | 0 | 2 days | Blood clot at base |
| 1 year | 0 | + | 0 | 0 | 0 | 0 | 60 days | Poisoned |

the sella turcica. After gently stripping the pituitary body from its stalk with a pair of fine right-angled forceps, a short free zone of the stalk can be clearly distinguished and divided by pinching the accurately approximating blades of the forceps (Fig. 4). The attachment to the posterior clinoid process can be separated by the same pinching process with the tweezer blades or by the sweep of an angled hook (Fig. 3). A little bleeding which accompanies the liberation of both attachments is easily controlled by two or three gentle applications of moist cotton.

TABLE II

*Total hypophysectomy in puppies*

| ESTIMATED AGE | CAUTERIZATION OF BASE | POST-OPERATIVE | | | | | DURATION OF LIFE AFTER OPERATION | NECROPSY FINDINGS |
|---|---|---|---|---|---|---|---|---|
| | | Glycosuria | Polyuria | Signs of pressure | Convulsions | Motor weakness | | |
| **Without intravenous salt** | | | | | | | | |
| 3 weeks | 0 | | | + | | | 1 day | Slight damage to cortex |
| 3 weeks | 0 | | | | | | 1 day | Slight damage to cortex |
| 3 weeks | 0 | | | | | | 1 day | Damage to cortex |
| 5 weeks | 0 | | | + | ++ | | 3 days | Haemorrhage |
| 5 weeks | 0 | | | + | + | + | 6 days | |
| 5 weeks | 0 | | | + | 0 | 0 | 13 days | |
| 5 weeks | 0 | | | | | | 1 day | |
| 5 weeks | 0 | | | + | | | 1 day | Blood clot |
| 5 weeks | 0 | | | + | | | 1 day | Small blood clot |
| 5 weeks | 0 | + | + | | | | 3 days | Killed in fall |
| 5 weeks | 0 | + | 0 | ++ | | | 2 days | Blood clot at base |
| 3 weeks | 0 | | | | | | 9 months | |
| **With intravenous salt** | | | | | | | | |
| 5 weeks | 0 | Trace | 0 | 0 | 0 | 0 | 10 days | Pneumonia. No evidences of damage to cortex or of haemorrhage |
| 5 weeks | 0 | Trace | 0 | 0 | 0 | 0 | 6 weeks | Died following administration of vermifuge. Marked emaciation. No haemorrhage |
| 7 weeks | 0 | | | + | 0 | 0 | 1 day | Died of haemorrhage following operation |
| 7 weeks | 0 | ++ | | | | | Still alive | Living and well 3 months after operation |
| 7 weeks | 0 | Slight | | | | | Still alive | Living and well 3 months after operation |
| 8 weeks | 0 | ++ | + | | ++ | Walks in circle | 8 days | Pneumonia following ether which was given to take x-rays |

The entire intracranial part of the operation can be completed in five to ten minutes and the wound left absolutely dry. It need not be added that bleeding must be absolutely checked, for even the slightest postoperative haemorrhage will compromise the life of the animal. Doubtless haemorrhage has also been an important factor in many of the results of experimental hypophysectomy—as in all human intracranial operations.

Minor differences in the anatomy of the hypophyseal region make the careful extirpation harder or easier, as the case may be. A falciform band of supporting tissue running from the base of the brain to the bridging carotid artery, a shorter hypophyseal stalk, and a posterior lobe attachment more concealed than usual in an anteriorly curving posterior clinoid process, are the variations which have some bearing on the greater difficulties in the performance of the operation.

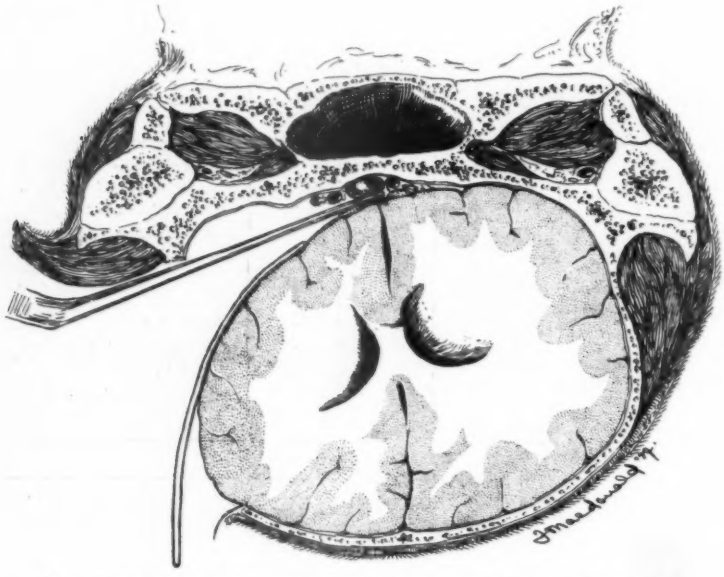

FIG. 5. DIAGRAM TO SHOW HOW THE PITUITARY REGION IS REACHED

The lateral wall of the skull is removed as far toward the base as possible. The brain volume is reduced by intravenous hypertonic salt solution.

### OPERATIONS ON YOUNG PUPPIES—SHRINKING THE BRAIN BY THE METHOD OF WEED AND MCKIBBEN

The operation as outlined above is satisfactory for dogs older than six months, but not for young puppies. In fact it is well nigh impossible to extirpate the hypophysis successfully in puppies from six to ten weeks of age.

The cause of death in puppies is precisely that in adults, namely, intracranial pressure resulting from injury to the brain, but there are two outstanding anatomical features of a puppy's brain which render it different from the adult brain and make the operative effects much more rapid and severe. The brain of a puppy is so soft that practically any pressure with instruments will cause destruction, and the amount of fluid which can be withdrawn from the subarachnoid space in an effort to obtain room for the operative exposure is far less, both relatively and absolutely, than in the adult. It is now possible to overcome these handicaps by making use of the brilliant discovery of Weed and McKibben,[18] that intravenous injection of concentrated sodium chloride solution withdraws fluid from the brain and causes it to shrink in an astounding manner and yet without any appreciable injury to the animal (or human being). The dose of hypertonic solution contains 0.33 gm. of sodium chloride per kilo of bodyweight. Half an hour later the volume of brain is so greatly lessened that with the hanging-brain method the hypophyseal exposure is better than that obtainable in the adult animal without the use of concentrated saline (Fig. 5). Since learning the great value of shrinking the brains of puppies, we have also uniformly adopted the method in adults and, needless to say, the approach is immeasurably easier and the results even better.

[18] Weed (L. H.) and McKibben: Pressure changes in the cerebrospinal fluid following intravenous injection of solutions of various concentrations. *Amer. Jour. Physiol.*, 1919, xlviii, 512; Experimental alteration of brain bulk. *Amer. Jour. Physiol.*, 1919, xlviii, 531.

### RESULTS OF TOTAL HYPOPHYSECTOMY

The results of these hypophysectomies are summarized in the accompanying tables. Of thirty-one adult dogs, ranging in estimated age from seven months to three years, five (5) died soon after operation from haemorrhage and cerebral trauma; seven (7) died in the country where they were sent for the summer, and autopsies were not obtainable; distemper caused the largest mortality, ten deaths occurring from 8 days to one year after operation. This seemingly excessive toll is, however, essentially similar for unoperated animals which were housed in the laboratory. In seven of the series the base of the brain was

FIG. 6. ADULT DOG 7 MONTHS AFTER TOTAL HYPOPHYSECTOMY AND CAUTERIZATION OF THE BASE OF THE BRAIN

The animal was poisoned a month later. At the time of his operation he was probably a year old and at the time of death was 4 pounds heavier. There was no apparent adiposity.

sectioned serially and studied for microscopic remnants of the anterior lobe, and in none were any found. In five of the seven so studied, the base had been cauterized at the time of operation. The duration of life in these seven histologically proven total extirpations was 13, 22, 27, 69, 90, 104 and 138 days. The presence or absence of polyuria, glycosuria, convulsions and evidences of motor weakness immediately after the operation, are included in the tables because they were part of our studies at the time. Some of these results, particularly of polyuria and polydipsia, will have a more direct bearing in a subsequent publication in which the functions of the hypophysis will be considered.

The table of operated young puppies, of three to eight weeks, presents a totally different picture. In the first twelve hypophysectomies in puppies, only one survived longer than two weeks. It lived for nine months. Its post-operative course was difficult and recovery slow. During the operation the temporal lobe was extensively injured and largely sacrificed. Without doubt its stormy convalescence was due to the cerebral injury, but doubtless also its life was preserved by the fact that the deliberate sacrifice of this injured brain prevented an increased intracranial pressure. The table of the post-operative life history of the first eleven puppies might well be that of similar tables in adults by Paulesco and others who claimed the hypophysis to be essential to life. The results are practically identical. But the series beginning with the use of Weed's hypertonic salt solution is

FIG. 7. TWO PUPPIES, 6 WEEKS OLD

The photograph was taken the afternoon of the operation at which the hypophysis was totally removed. The hypophysectomized puppy on the left, by turning, has obscured the operative wound which is on the right side of the head.

entirely different, because the brain is spared the cerebral trauma incident to the operation. These results are, then, totally at variance with those of the earlier group of puppies and quite like those of the adult dogs—as far as life is concerned. On the day of the operation the animals walk about and on the following day they are almost as playful and lively as the controls (Fig. 7).

In order to insure total absence of microscopic remnants of the anterior lobe at the base of the brain, the stump of the infundibulum (to which cells might remain attached) and the region immediately contiguous have been well cauterized with a platinum tip in thirteen adult dogs. The general post-operative course of these animals (possibly excepting polyuria) has not been unlike that of those without cauterization. Microscopic studies (serial sections) of the base of the brain in many of the surviving animals have been made and the sections have been uniformly negative for anterior lobe cells.

With painstaking care and attention to detail there should be a minimal operative mortality in animals over six months, and while our later series of young puppies has been small (six), there seems no reason why the results should be greatly different at this age. All deaths following soon after operation are explainable as the result of trauma, haemorrhage or pneumonia, and deaths appearing later can be readily explained on well-recognized pathological grounds.

SUMMARY

An operative procedure is presented for the safe removal of the canine hypophysis.

FIG. 8. PHOTOGRAPH TAKEN OF CONTROL PUPPY (LEFT) AND HYPOPHYSECTOMIZED PUPPY (RIGHT), 7 WEEKS AFTER TOTAL REMOVAL OF HYPOPHYSIS

The operation was performed when the puppy was 6 weeks old.

We have found no evidence to support, and every evidence to refute, the assumption that the hypophysis is essential to life, or that the group of symptoms which have been described by others as preceding death in hypophysectomized animals are of hypophyseal origin.

# The Normal Rhythm of the White Blood Cells

By F. R. Sabin, R. S. Cunningham, C. A. Doan, and J. A. Kindwall[1]

*From the Department of Anatomy, The Johns Hopkins University*

THE fact that there is a fairly constant number of white cells in a cubic millimeter of blood, and that there are variations of this number characteristic of different types of disease, has become common medical knowledge since the methods for counting blood-cells were introduced. There is indeed nothing in the entire animal economy more amazing than the fact that, from hour to hour and from day to day, the number of the white cells remains so remarkably constant. It is wholly obvious that the mechanism involved in the maintenance of this remarkable condition must be one of great importance and it is hoped that the present communication may assist somewhat in inaugurating a better understanding of the physiology of the white blood-cells.

Through the use of the so-called supra-vital technique we have found that the number of the blood-cells varies in a definite rhythm; and we furthermore believe that the supra-vital technique was necessary for this study because it is only through it that it has so far been possible to obtain any estimate of the amount of cell-death, which we have found to be a factor essential to the analysis of the rhythmical changes in the counts of the white blood-cells.

After we had analyzed the counts which we had made on the supra-vital preparations, we found it possible to correlate our results with the data obtained by counting the corresponding fixed films, but we were able to obtain this correlation only by including all the different types of degenerations of the leucocytes, which have heretofore been unanalyzed and for the most part disregarded, and which have been considered as due to errors in technique rather than to different conditions in the cells themselves.

We have followed the general classification first clearly established by Naegeli (28): that there are three main groups of the white blood-cells, the granulocytic leucocytes, perhaps better termed "granulocytes," the "lymphocytes," and the "monocytes." In the supra-vital differential counts, we have classified the cells under nine headings. We have followed the usual custom and divided the granulocytes into three types depending upon the staining of their granules; *i.e.*, into neutrophiles, basophiles, and eosinophiles. In the case of the lymphocytes, we have found it

[1] Mr. Kindwall's participation in this problem was made possible by a grant received from the Henry Strong Denison Medical Foundation.

an advantage to classify them under three forms, according to their size; as small, intermediate, and large forms. It may be that these three types are simply different stages in the life cycle of a single type; or in other words, this classification might be compared to the Arneth system of determining the different degrees of maturity and senility of the neutrophilic leucocytes by means of the lobulation of the nucleus. Nevertheless, the young, mature and senile stages of lymphocytes are still so little analyzed, that it is thoroughly worth while, for purposes of study, to subdivide the circulating lymphocytes as much as possible. We have found, in the supra-vital technique, that the large mononuclear cells and transitionals make one group, namely, the monocytes, so that we have discarded entirely the double classification, as will be brought out later. Besides these seven groups of viable cells we have found that there are large showers of dying neutrophilic leucocytes, which are always characteristic; and a very small group of damaged cells, which we think are lymphocytes undergoing disintegration. Both of these groups must be taken into consideration in establishing the fact of a normal rhythm of the blood-cells.

By the supra-vital technique, we mean the study of living blood-cells, differentiated by means of their reactions to very small doses of certain dyes. The technique has been given by Sabin (34).

We began our studies, concerning the rhythm of the leucocytes, with the consideration of the type of cells which we have called the "non-motile leucocyte." The name "non-motile leucocyte" was given to these cells by Sabin (34) in 1923. In studying living blood-cells she noted that there were leucocytes which had lost their characteristic property of motility, and because of this change she gave them the name "non-motile leucocyte." We now know that the essential change in this cell is not merely a loss of motility, because we have seen this type of change in the granules of the myelocyte, which is a cell that has not yet developed the characteristic type of ameboid movement. The transformation of leucocytes into this phase is essentially a chemical and physical change in the neutrophilic granules. As seen in the living preparations, the cell first stops moving and rounds up, and the granules no longer stain with neutral red. It is obvious that these granules have either lost all power of reacting to vital dyes, or that the cell membrane has become impermeable to the dye. We think that the granules have actually lost their power to react to the dye because, when we find the membrane ruptured and the granules free in the surrounding fluid, they are entirely unstained. It is by their high refractivity that they are readily recognized in the supra-vital preparations; they are decidedly more refractive than the granules of the eosinophilic leucocytes. In correlation with this physical change, the granules have changed chemically, for their solubilities are different. The fact that the granules in this state are soluble in alcohol, while the neutrophilic granules are not, has an important bearing on the analysis of these cells in films of

blood, stained by the modern methods that involve fixation in alcohol.

This cell was discovered by Schilling (37) in 1908. He described its development from a living leucocyte, seen under dark field illumination. Schilling observed the cessation of motility and also described the subsequent disappearance of the centrosphere; in his preparations he found that the striking point was that the neutrophilic granules spread farther apart and extended out into the ectoplasm, which in the living cell is always free from granules. The change in size and in refractivity, which is so striking in our type of preparation, was evidently not so obvious under dark field illumination. Nevertheless, in Schilling's figures the granules are shown as markedly larger than neutrophilic granules. Schilling referred to this cell in what we deem a significant phrase, namely, that it represented the "physiological death of the leucocyte." He also noted, as have we, that the nucleus, which had been polymorphous, may become practically round, and this change we have considered as probably in the nature of an edema. This type of cell was illustrated by Schilling (37) as cell Abb. $11^2$ on page 441, and by Sabin (34) in Fig. 2 on Plate XXXV.

Schilling also described two other forms of degenerating leucocytes, one, a form which he found in saliva and interpreted as a very young leucocyte. In our judgment, this cell was probably a myelocyte, which had undergone degeneration. This cell had a small centrally-placed, round nucleus with a rim of clear cytoplasm around it and a rim of granules in the edge of the cell. He also described fragments of old leucocytes containing bits of nuclear material. We have also seen these fragments of the cytoplasm of leucocytes in our preparations; indeed, we have often watched their formation. When a leucocyte is moving on the coverslip, it very often puts out excessively long processes such as the one shown in Sabin's Fig. 1, Plate XXXV (34). In this cell there is shown a tiny swelling at the end of the long pseudopod and it is such bits of cytoplasm as this that are frequently left behind as the cell moves. The dropping off of such bits of cytoplasm does not seem to injure the cell in the least, but that the bit of abandoned cytoplasm begins immediately to undergo changes is shown by the development of a Brownian movement of the particles, entirely different from the streaming of the cytoplasm characteristic of the intact cell. The glittering of which Schilling speaks in regard to these fragments may have been due to the Brownian movement as seen under dark field illumination. We have only very rarely seen nuclear fragments in these bits of cytoplasm, but they all contain stainable neutrophilic granules. Brownian movement of the particles which is so constant a phenomenon in these fragments of leucocytes, when first formed, may develop in the cell itself, but we interpret it as a sign that the cell has been damaged and has taken in fluid from the surrounding medium. We have seen leucocytes showing marked Brownian movement of the particles in certain cases of infection and in serum sickness. Complete fragmentation of the

neutrophilic leucocytes may occur, but is rarely seen in films of normal peripheral blood.

We first noted in making repeated supra-vital films of blood from the same individual that the cell we have called the "non-motile" leucocyte occurred quite irregularly; at times the preparations contained none, and again these cells appeared in numbers that seemed to us high proportions. Since it was then obvious that these dying cells appeared in showers, we determined to undertake a more careful survey of their occurrence. It is a generally accepted biological theory that in the adult organism, which is neither gaining nor losing weight, the rate of cell-division is correlated with the rate of cell-death. On this basis, it seemed plausible that, if we could arrive at an estimate of the amount of cell-death of the leucocytes in the blood-stream, we might open up some method of analyzing the rhythm of the leucocytes. It is, of course, clear that the analysis of this rhythm is an exceedingly complex problem, and that there are obviously a large number of factors to be considered. Of these we have felt that there were two major groups. The first of these groups involves the whole mechanism of the delivery of mature cells into the circulation. Here we must consider that all of the blood-cells throughout life have to differentiate from very primitive types of cells. In this process, there is, first, the continued division of immature cells, the progressive maturation and continued division of these immature types, the division of more mature forms and, finally, the forces that bring the cells into the circulation. The complete analysis will also involve finding out why, under normal conditions, only the mature types enter the blood-stream, while, under abnormal conditions, somewhat and even extremely immature types are found in the blood-stream. The second group of factors includes the question of the passing of the cells out of the circulation under varying conditions as well as the possible marked shifting of the proportions of the different types of cells in the visceral as contrasted with the peripheral circulation, according to varying functional requirements. It is clear that both of these great groups of factors are complex.

In our studies of the rhythms of the leucocytes, we first made counts of human blood at irregular intervals; but we soon became convinced that, to be of any significance, the interval of time must be as short as possible and also entirely regular. We have found an interval of 15 minutes to be feasible. Much might have been gained by a shorter interval, namely, one of 5 to 10 minutes, since we have reason to believe that the showers of the non-motile cells are very transient but we were unable, with our group of workers, to make the supra-vital differential counts at a shorter interval than 15 minutes.

We are reporting six experiments at this time. In the first five series we made, on each individual, a count of the total number of red cells, of the total number of the white cells, a supra-vital differential count of 100 cells, a count of the platelets and of the reticulated red cells, every 15 minutes for 7 to 8 hours. We

also pulled a pair of coverslips at each interval for differential counts on fixed films. The counts of the reticulated red cells were made for us by Dr. Albert Key; the counts of the platelets were made by Mr. J. Warner Duckett; the rest of the data were obtained by the authors. The studies of the red cells, of the reticulated red cells, and of the platelets have all shown definite rhythms, but it is only the rhythms of the white cells that we are reporting at this time. The one curve (Chart 4 D) which shows the rhythm of the red blood-cells is given for a specific control, and not in any attempt to analyze completely at this time our results on these cells. The observations which we are reporting here are all from human blood; we have made many more such experiments on the blood of rabbits which show the same general rhythms, so that we feel more confident in reporting these data from a limited series. For Chart 6, we studied only the white blood-cells and we were also assisted in making the counts by Dr. S. Sugiyama, of the Kyoto Imperial University, Japan. In this series we were able to make a supra-vital differential count of 200 cells every 15 minutes. One hundred cells were counted on each of two films and the average taken.

In all of these series we have attempted to maintain as rigid a technique as possible. The individual whose blood was being counted has in every case been in as uniform an environment as it was possible to maintain. In three of the cases, the individual has taken an active part in the work of the experiment. In 5 cases (Charts 1 to 5) the subject has refrained from food and water from the time of dinner the night before the experiment. The individual of Chart 6F drank a pint of milk for breakfast three quarters of an hour before the experiment began. None of the others had any breakfast. Four took no lunch whatever. The individual whose blood is shown on Chart 2 B drank a glass of water at 12:15 and three quarters of a pint of cream at one o'clock; and the subject whose blood is shown on Chart 4 D had a lunch of proteid (beefsteak) at 12. The others took no food throughout the experiment. All of our charts show practically the same type of rhythm whether food was taken or not. The subjects refrained from drinking water from the time of dinner the night before, so as to limit as much as possible the changes due to variations in blood-volume; the individual of Chart 6 F who had a pint of milk for breakfast, and especially the subject of Chart 2 B, who had the glass of water and the three quarters of a pint of cream, had certainly an amount of fluid sufficient to compensate for the excretion of the kidneys during the 7 to 8 hours of the experiment. Five of the counts were made on men, one on a woman.

In the studies we have made of the rhythms from the blood of rabbits, we have obtained venous blood from the ear; for human blood, we have used the fingers, and employed an automatic blood sticker set for a puncture deep enough to give a free flow of blood, a flow which we consider to be a mixture of venous and capillary blood. We have tried for a flow free enough to require

no external pressure whatever to aid it; a different finger was used for each successive puncture, and we did not use the same place on any one finger twice during the same day. We wiped off the first drop, then took preparations for the supra-vital differential counts, then for the total counts, and finally the films for the fixed preparations. In the first five series the specimens for the platelets were then taken and, finally, the films for the reticulated red cells. In the sixth experiment we took the specimen for the total white count first, then a supra-vital preparation, next two covers for fixed films, then another supra-vital preparation, and, finally, a second pair of coverslips for fixed films. Throughout each experiment we have used the same pipette for diluting the blood; for the first five charts we used the same side of the counting chamber; for the sixth experiment both sides of the counting chamber were filled and the average of the two counts taken.

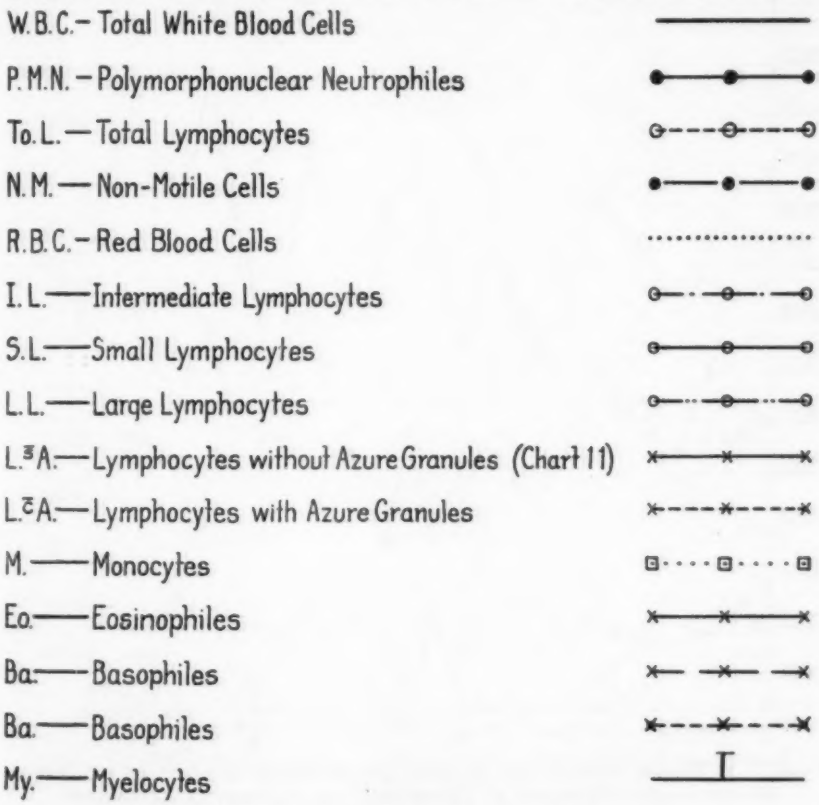
W.B.C.– Total White Blood Cells
P.M.N. – Polymorphonuclear Neutrophiles
To.L. — Total Lymphocytes
N.M. — Non-Motile Cells
R.B.C. – Red Blood Cells
I.L. — Intermediate Lymphocytes
S.L. — Small Lymphocytes
L.L. — Large Lymphocytes
L.ᵃA. — Lymphocytes without Azure Granules (Chart 11)
L.ᶜA. — Lymphocytes with Azure Granules
M. — Monocytes
Eo. — Eosinophiles
Ba. — Basophiles
Ba. — Basophiles
My. — Myelocytes

Fig. 1. Conventions for the Charts

For the supra-vital counts the slides and coverslips were scrupulously clean; the slides were all prepared at one time, so that the cleaning was entirely uniform and, furthermore, all that were used in any one experiment were coated with neutral red from the same solution. This care in technique was observed in order to prevent any avoidable error in the supra-vital counts. For these experiments, in which we were testing especially the fact of showers of non-motile cells, we used films of blood that were consistently thicker than those desirable for fixed preparations. In some preliminary studies made by Mr. Kindwall, it appeared that the non-motile cells tended to be more numerous in thin places in the

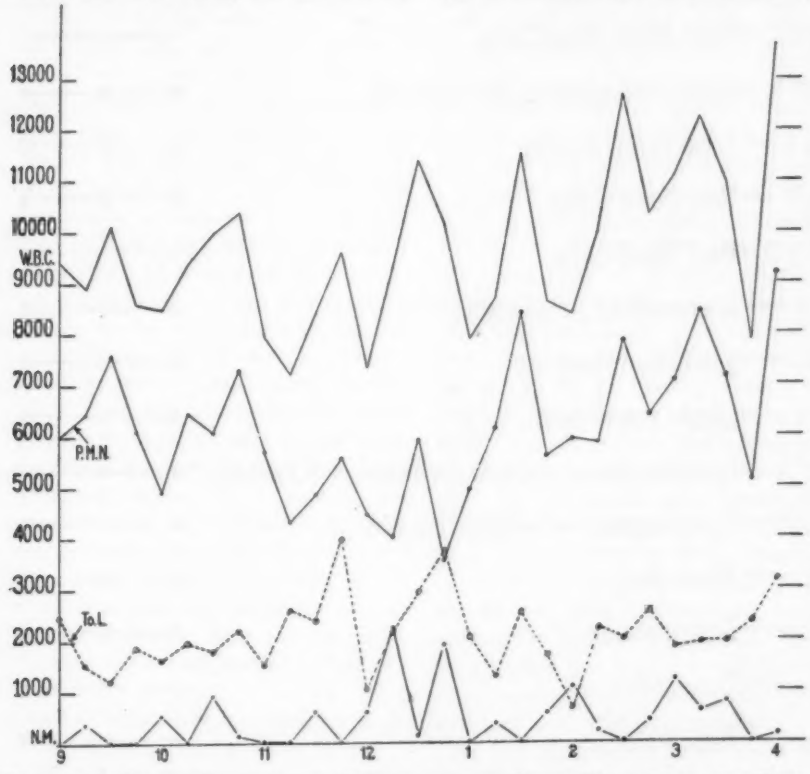


CHART 1 A. CURVES SHOWING THE NUMBERS OF THE WHITE BLOOD-CELLS, THE POLYMORPHONUCLEAR NEUTROPHILIC LEUCOCYTES, THE NON-MOTILE LEUCOCYTES AND THE LYMPHOCYTES OF INDIVIDUAL A

The differential counts were made from supra-vital films, 100 cells being counted at each interval. The hours are given on the abscissae and the numbers of cells on the ordinates. The individual took neither food nor drink from the time of dinner the night before throughout the time of the experiment.

films, so that we tried for films in which there was a slight overriding of the red blood-cells. We have never used any films in which the red cells were in rouleaux, but by having some overriding of the red cells we endeavored to avoid the difficulty of an undue pressure of the coverslip on the white blood-cells. In these thicker preparations we have recorded whether each non-motile cell was isolated in the field or was so near a motile cell as to leave little doubt that it had not been caused by the pressure of the coverslip. We think it a fair assumption that, since so large a proportion of the non-motile cells were near motile forms on our preparations, the pressure of the coverslip can not be considered as a major factor in their production. There is, however, a considerable tendency for the non-motile cells to occur in clumps, at times even in agglutinated masses. On a given slide there may

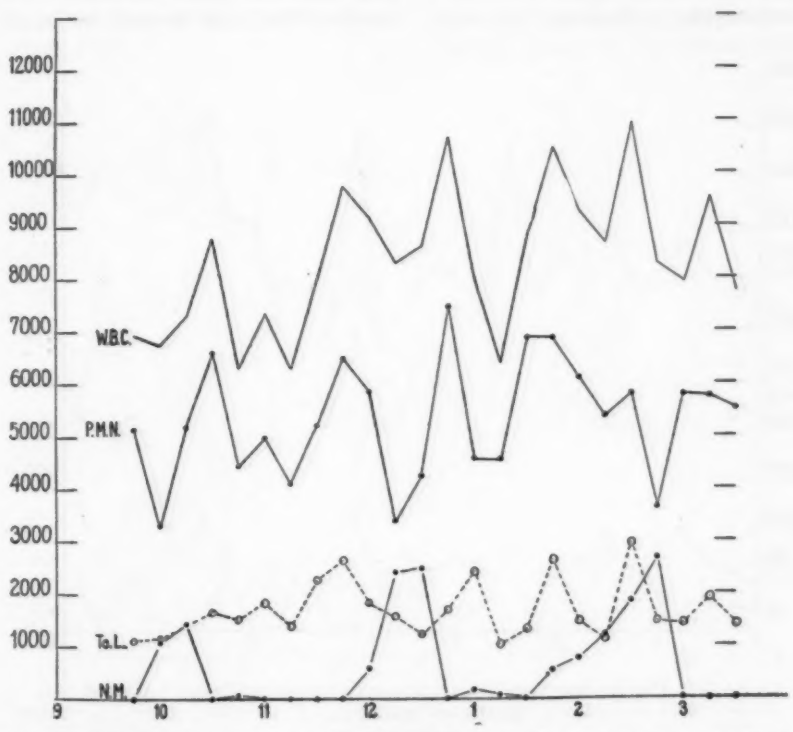


CHART 2 B. CURVES SHOWING DATA SIMILAR TO THOSE OF CHART 1 A, FROM THE BLOOD OF INDIVIDUAL B

This person took neither food nor drink from the time of dinner the night before up to 12:15, when a glass of water was taken, followed by ¾ pint of cream at one o'clock.

be many of them in one part of the preparation and few in another. Moreover, we have repeatedly noted the non-motile forms sticking to clumps of platelets. It is certainly possible that cells in our preparations are hastened into the non-motile phase by being drawn from the blood-stream; furthermore, the supra-vital preparation may suffer some increase in the type during the fifteen minutes of the count, but we feel confident that this factor of error is a slight one and that our supra-vital technique can be taken as giving a fair estimation of the number of neutrophilic leucocytes in the circulation which are dying or about to die at a given moment. In the supra-vital preparations we counted the cells as near the edge of the films as possible, but we could not use the extreme edge since the immersion oil would then mix with the vaseline, and make an opaque medium. We mention this point because we found it advisable not to limit the counting of the fixed films to the center of the preparations, since there is a tendency for the larger forms to occur near the edges and these larger forms include the cells which we call non-motile cells in the supra-vital films, and, of course, the monocytes.

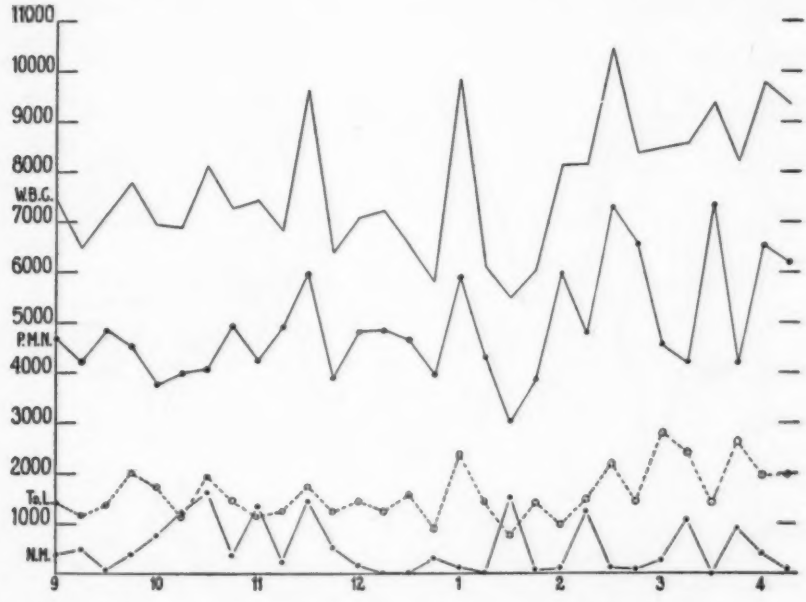


CHART 3 C. CURVES SHOWING DATA SIMILAR TO THOSE OF CHART 1 A, FROM THE BLOOD OF INDIVIDUAL C

This person took neither food nor drink from the time of dinner the night before throughout the time of the experiment.

Finally, it seems to us to be wholly obvious that even if the physical factors involved in the preparation of the supra-vital smear assist in the production of non-motile cells, it is still a relative measure of the fragility and death-rate of the cells, since there are always wholly normal living cells intermixed with these dying forms.

of the number of cells from these data. Not many observers have counted the blood of the same individual more than two or three times a day, though here and there a few observers have made more extensive and elaborate studies. These studies have, however, established the average number of the various types of the white blood-

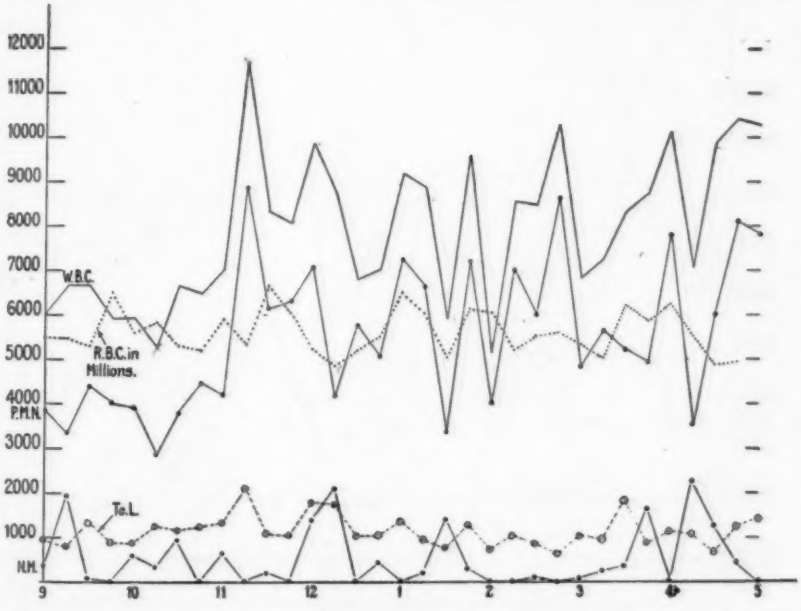


CHART 4 D. CURVES SHOWING DATA SIMILAR TO THOSE OF CHART 1 A, FROM THE BLOOD OF INDIVIDUAL D

This person took neither food nor drink up until 12 o'clock when a lunch of beefsteak was taken. The line of red blood-cells to be read in millions is added to this chart.

### LITERATURE

The normal number of the white blood-cells has been investigated very many times, but usually the method has been to take one or more counts from a series of individuals and then to calculate an average, normal level

cells and have given us a mean level from which to estimate any very abnormal deviations. Naegeli, Arneth, Galambos, Türk, and others have reviewed the literature dealing with this average mean level so thoroughly that it will not be neces-

sary to refer in detail here to all the separate publications. The borders of normality are variously estimated, but always correspond within a fair limit.

Torday (41) found in 61 cases that the lowest count was 3130 and the highest 9800, the average being 6700. In his cases the average for the polymorphonuclear neutrophiles was 65.5 per cent, that for the lymphocytes, 27.6 per cent and for the transitionals, 0 to 8 per cent.

Galambos (16) found that in healthy individuals the normal number of white blood-cells varied from 3500 to 12,500 with an average of 7613. He also found that in the same individual the number of white blood-cells showed variations at different periods during the day amounting to almost 100 per cent.

Türk (42) gives the normal as from 5000 to 10,000 white blood-cells with an average of 6000 and in nervous individuals an average of 7000.

The great clinical significance of leucocytoses has made the study of the mechanism of the increase and decrease in the number of the white

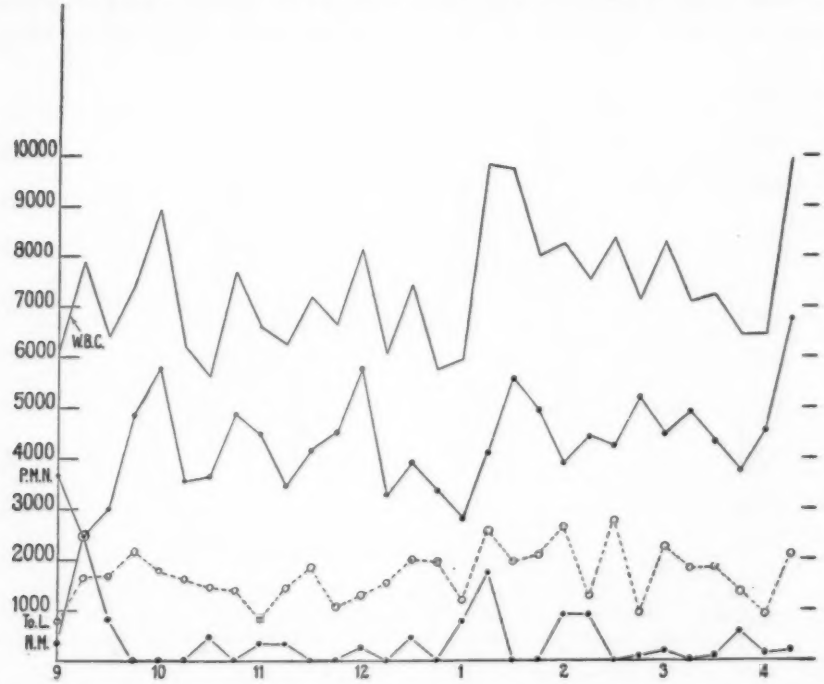


Chart 5 E. Curves Showing Data Similar to Those of Chart 1 A, from the Blood of Individual E

This person took neither food nor drink from the time of dinner the night before throughout the experiment.

blood-cells one of the greatest importance, and many observations have been reported in regard to the mechanism by which the number of these cells is increased and decreased. Many of these observations have dealt with pathological states, or with conditions which cannot be considered as strictly physiological owing to various experimental procedures, but these are of importance in helping to elucidate the normal mechanism of the rhythm of the cells. Many investigators have found that the injection of foreign proteins, bacteria, drugs, etc., produces a definite and very prompt leucopenia followed by a leucocytosis. Among those who have described such changes Arneth (1) has perhaps done the most extensive and detailed work. He has studied the white blood-cells after many such experimental procedures and has concluded that there was a widespread destruction of cells during the period preceding the leucocytosis and that these cells were replaced by the entry of young cells into the circulation from some of the hematopoietic centers. His method of study was to count the number of lobes in the polymorphonuclear neutrophilic leucocytes and to estimate the age as directly proportional to the number of lobes. Using this criterion he found a definite reduction in the number of the lobes during the leucocytosis follow-

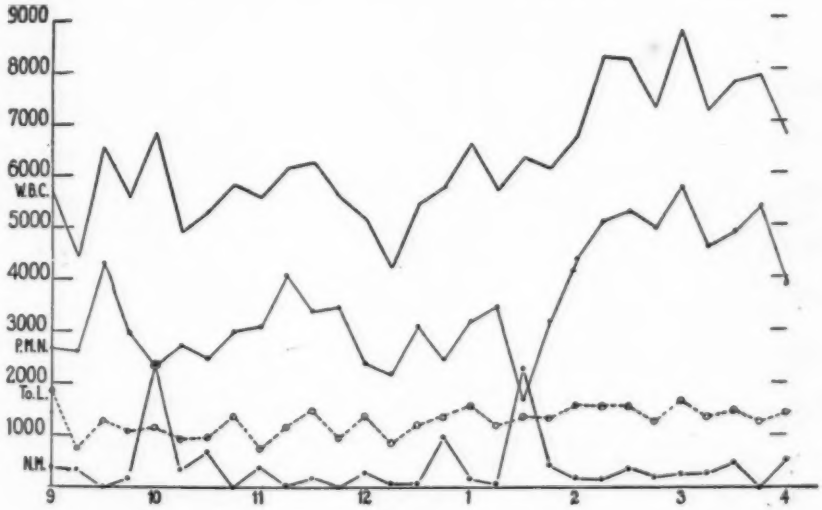


CHART 6 F. CURVES SHOWING DATA SIMILAR TO THOSE OF CHART 1 A, EXCEPT THAT THE SUPRA-VITAL DIFFERENTIAL COUNTS INCLUDED 200 CELLS, FROM THE BLOOD OF INDIVIDUAL F

This person took neither food nor drink from the time of dinner the night before except a pint of milk at 8:15 in the morning.

ing, in turn, the leucopenia after the administration of peptone or nuclein.

These observations of Arneth, supported by the similar work of Aschenheim (3) and others, on the effect of the injection of foreign substances, led to a controversy on the question of whether the cells were destroyed or merely temporarily removed from the circulation during the period of the leucopenia. Goldscheider and Jacobs (17) thought that the cells were retained in the vessels of the viscera and were not destroyed. Their evidence was largely based upon the histological study of sections cut after the production of the changes in the blood. We have referred elsewhere in this communication to our own observations on this phenomenon.

Many authors believe that the changes in the number of white blood-cells can be entirely explained by variations in the blood pressure and vaso-motor activities. Numerous experiments have been carried out with the object of influencing these factors by means of the injection of drugs or by the mechanical stimulation of the vaso-motor nerves. For example, it is well known that there is a leucocytosis following the administration of adrenalin and it is of interest to determine what relation this increase in the number of the blood-cells bears to changes in blood-volume or blood pressure.

Camus and Pagniez (8, 9) have shown that variations in blood pressure are followed by changes in the number of blood-cells, a lowering of

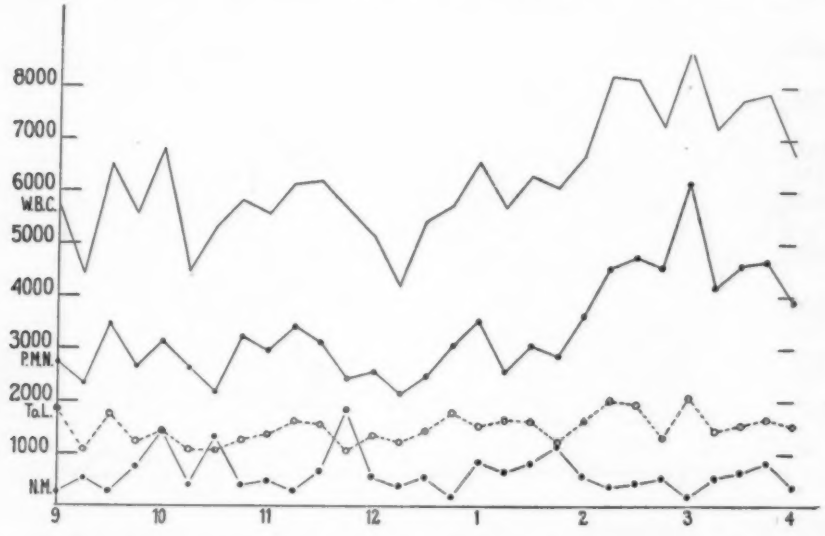


Chart 7 F. Curves Giving the Same Data as in Chart 6 F, Individual F, Except that the Differential Counts Were Made from Fixed Films Stained with Wright's Blood-stain

the pressure being followed by a decrease in the number of the cells. In their experiments they found that lowering of the blood pressure by stimulation of the pneumogastric nerve was followed by a definite and immediate decrease in the number of the white blood-cells. This leucopenia was evident in arterial and venous blood alike and was not confined to the peripheral blood. When the blood pressure was increased by injections of adrenalin or excitation of the sciatic nerve, there was no immediate hyperleucocytosis; the leucocytosis appearing only after the hypertension produced by the adrenalin had already begun to wear off. They think that a possible explanation for the difference in the behavior of the red and white blood-cells, which they found in their experiments, is that the latter can stick to the walls of the vessels and are more likely to do so if, for any reason, the blood flow is slow in the vessels, thus failing to sweep them into the current.

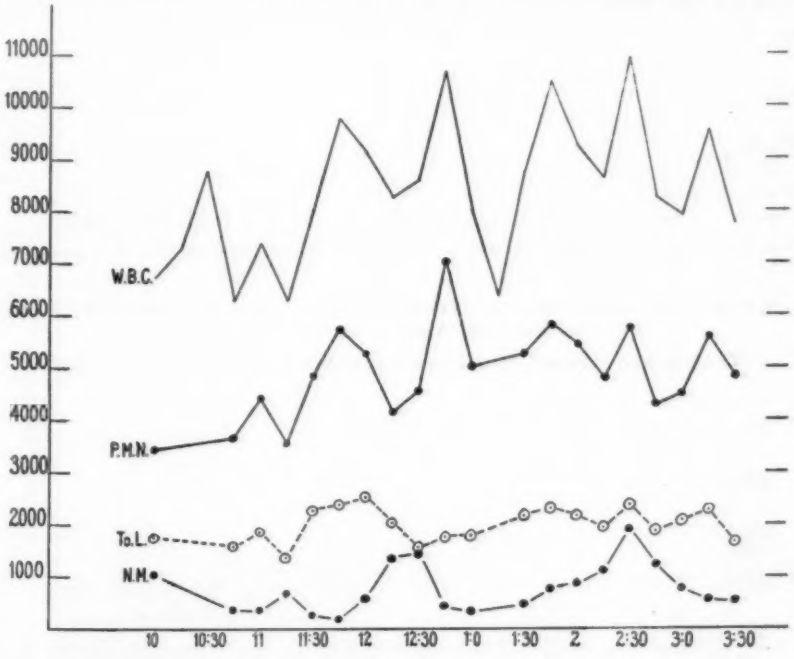


Chart 8 B. Curves Giving the Same Data as in Chart 2 B, Individual B, Except that the Differential Counts Were Made from Fixed Films Stained with Wright's Blood-stain

Zuntz and Vogel (43) found that injections of pilocarpine, given to dogs in doses of one milligram per kilo of body-weight, caused a marked general leucocytosis within 15 to 30 minutes. The same they found to

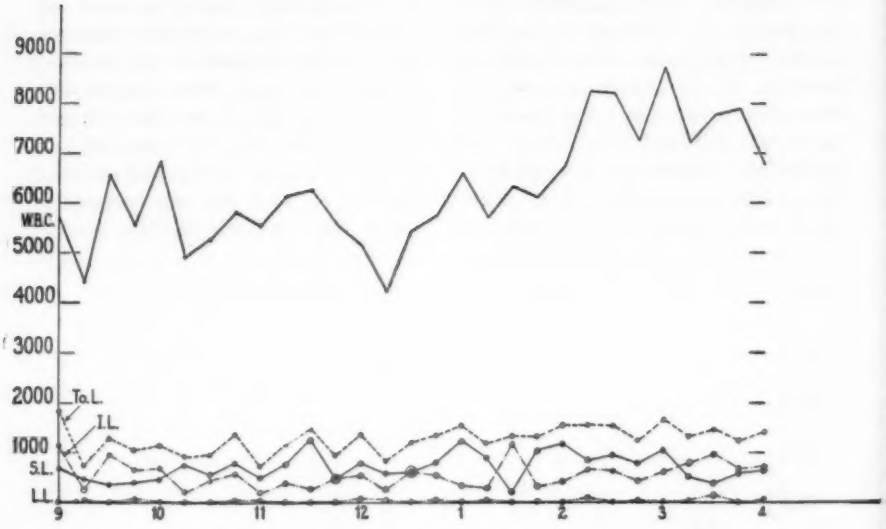


CHART 9 F. CURVES SHOWING THE THREE DIFFERENT GROUPS OF LYMPHOCYTES PLOTTED ON THE SAME SCALE AS CHARTS 1 TO 6

The data were obtained from the supra-vital differential counts of individual F

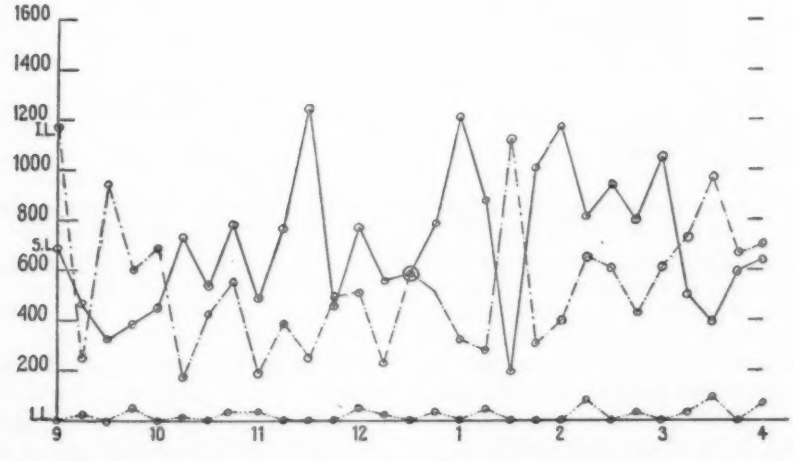


CHART 10 F. CURVES SHOWING THE LYMPHOCYTES FROM THE SAME DATA AS IN CHART 9 Y, PLOTTED IN HUNDREDS INSTEAD OF THOUSANDS. INDIVIDUAL F

be true in man, but to a less degree owing to the much smaller doses given. Extirpation of the spleen caused no change in the blood picture following the administration of pilocarpine, but when atropine was added to the pilocarpine, in either normal or splenectomized animals, there was practically no leucocytosis, demonstrating the antagonistic influence of in the number of leucocytes, whereas heating the finger caused an increase. Arneth (1, page 52), Becker (6) and others have found that there is a definite increase in the number of white blood-cells after cold baths, while Hasselbalch and Heyerdahl (19) found that sudden changes from a recumbent to an upright position or *vice versa* caused a rapid and marked

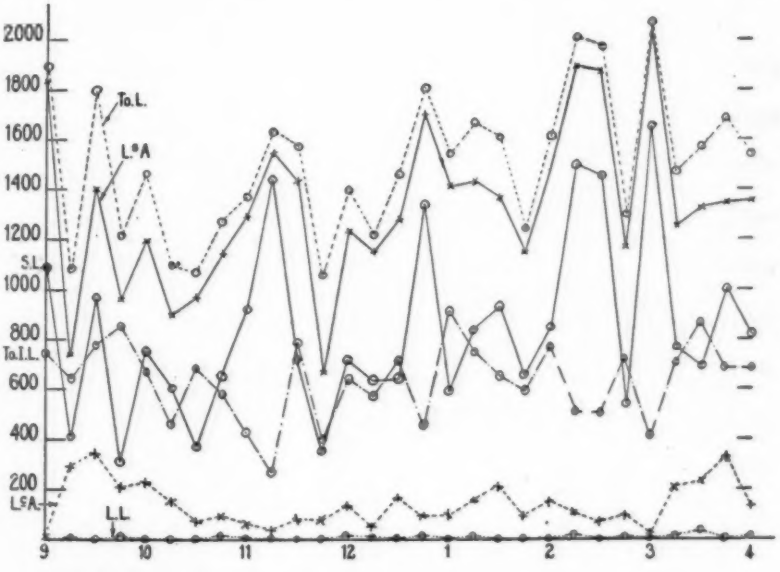


CHART 11 F. CURVES SHOWING THE LYMPHOCYTES FROM INDIVIDUAL F, PLOTTED FROM DIFFERENTIAL COUNTS TAKEN FROM FIXED FILMS STAINED WITH WRIGHT'S BLOOD-STAIN

these drugs on the white blood-cell picture.

Tinel and Santenoise (40) found that intense cooling of a finger brought about a lowering in the number of white blood-cells when counts were made on blood taken from that area. A stimulation of a mixed nerve also caused a decrease variation in the number of leucocytes. Their work was amplified by Ellermann and Erlandsen (15), who carried out a series of experiments on sudden changes in posture in normal individuals and on a few who were convalescing from some minor disorder, usually digestive. Blood counts were made just prior to the

change in position and of 23 cases studied all showed a positive reaction. The second blood count was made 10 to 15 seconds after the change in position had been accomplished, and in one and a half minutes it was found that the reaction had practically disappeared. The increase in pulse rate following the change in posture was marked in all cases.

to see that respiration was spaced regularly. It was found from these experiments that muscular work caused a constant increase of leucocytes up to 19–42 per cent, and that this disappeared in the course of 2 to 3 minutes but after a short while could reappear again on renewed exercise. This increase in the number of the leucocytes after muscular

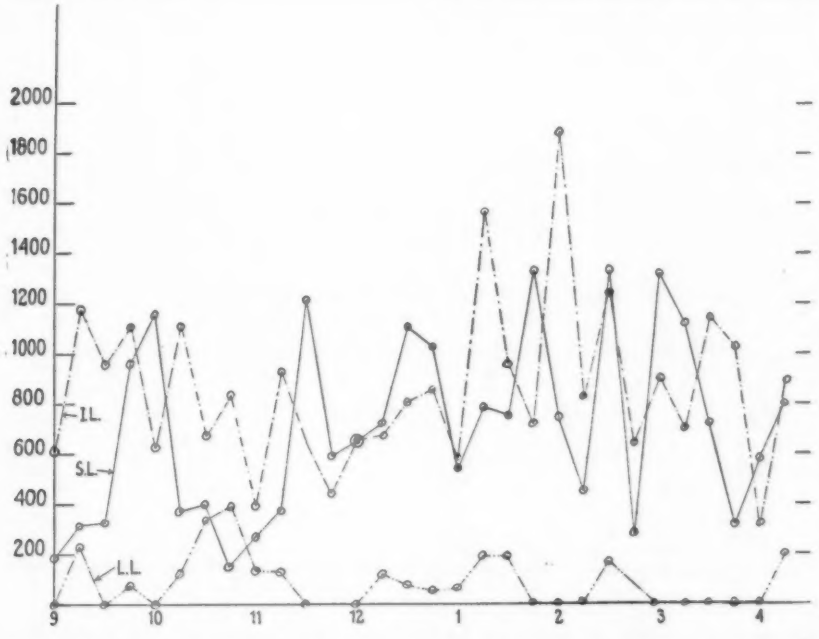


CHART 12 E. CURVES SHOWING THE LYMPHOCYTES FROM INDIVIDUAL E, FROM SUPRA-VITAL DIFFERENTIAL COUNTS

Ellermann and Erlandsen also carried out experiments to determine the effect of muscular exercise on the leucocytes. Men were left in a standing position holding a ten-pound weight over their heads as long as possible, or else they raised and lowered a ten-pound weight as long as they could, care always being taken

work seemed to be of the same character as that following change of position, *i.e.*, in amount and duration. In connection with some other work these investigators noticed that the drop of blood obtained immediately after the puncture contained considerably more leucocytes than the later ones. They carried out ex-

periments so arranged as to exclude coagulation phenomena and found that in nervous, anxious, fearful individuals the difference between the number of white blood-cells in the first drop and in later drops was much greater than in phlegmatic, apathetic individuals in whom the difference was very slight. They concluded from these findings that this difference (1 to 2 minutes after the injection). From their study of the literature, as well as from the results of their own work, Ellermann and Erlandsen conclude that the leucocytes find their way into the blood-stream in increased numbers because of increased heart activity and the subsequently greater rapidity of blood flow which sweeps them from the

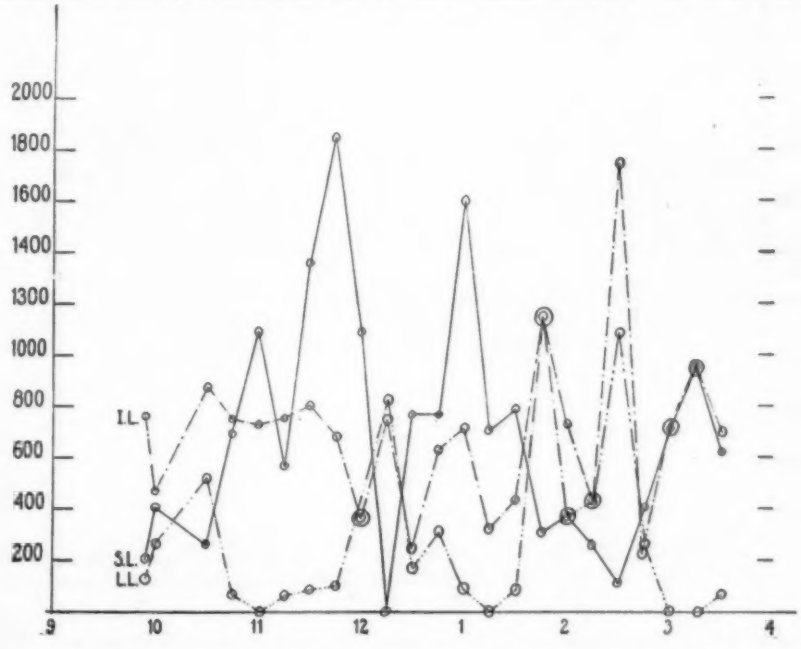


Chart 13 B. Curves Showing the Lymphocytes from Individual B, Taken from Supra-vital Differential Counts

was due to the excited condition of the patient, and that the experiments all indicated that increased heart activity was the chief factor involved. In order to test the question of heart activity they studied the effect of strophanthine on rabbits. In all cases a marked increase in the number of leucocytes took place very rapidly walls of the vessels into the current. The chief factor they consider to be mechanical.

Similar conclusions with regard to the effect of muscular exercise and heart activity on the white blood-cells have been reached by Becher (5), Grawitz (18), Hochstetter (20), and others. Becher (5), after muscular

exercise, found an increase in the number of leucocytes which appeared in 15 to 20 minutes and returned rapidly to normal again. Becher believed this leucocytosis to be due to the migration of ripe cells from the reserve depots of the various organs, the migration being stimulated by certain mechanical and circulatory factors.

after the ingestion of food, but this has been questioned by a number of observers.

Pohl (29, 30) studied digestive leucocytosis in dogs after 18 hours of fasting and found after feeding them meat that they had a leucocytosis beginning in one hour and reaching a maximum at three hours. There was no reaction in a few older and

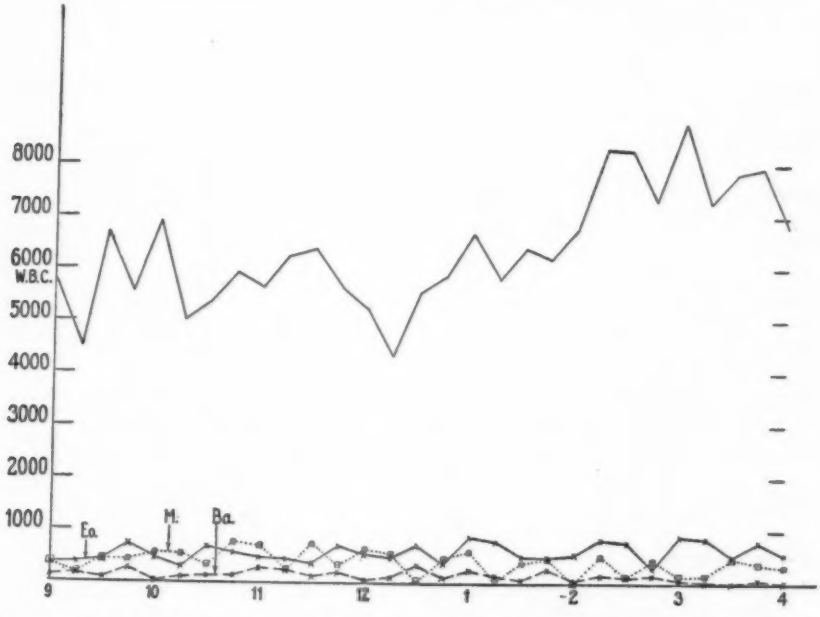


CHART 14 F. CURVES SHOWING THE EOSINOPHILIC AND BASOPHILIC LEUCOCYTES AND THE MONOCYTES PLOTTED ON THE SAME SCALE AS CHARTS 1 TO 6 FROM THE SUPRA-VITAL DIFFERENTIAL COUNTS OF INDIVIDUAL F

Among the most discussed and most studied phases of the variations in the number of white blood-cells is that following the taking of food, the so-called digestive leucocytosis. The majority of authors agree that a more or less marked change in the number of leucocytes does take place

over-nourished dogs. With non-albuminous foods he obtained no leucocytosis. These findings were substantiated in studies on man, and the leucocytosis was especially marked if there had been a long preliminary fasting period.

Rieder (32) confirmed Pohl's find-

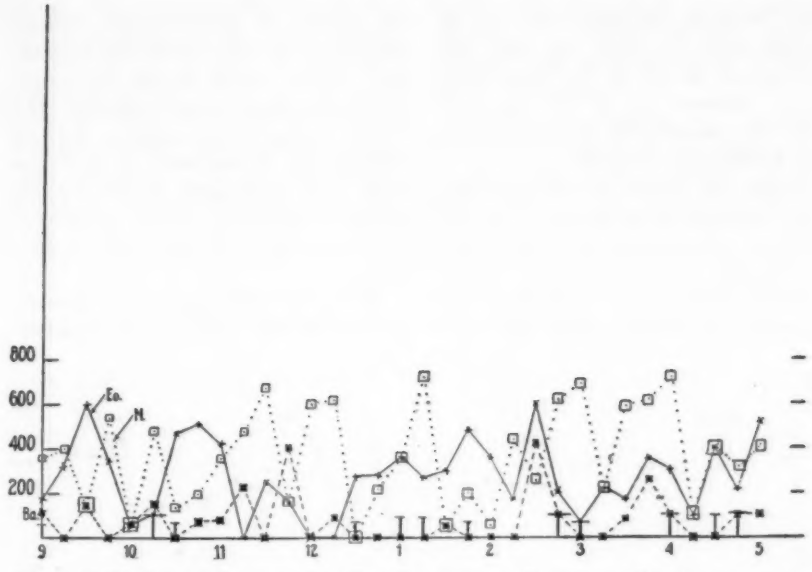


CHART 15 D. CURVES SHOWING THE EOSINOPHILIC AND BASOPHILIC LEUCOCYTES, THE MONOCYTES AND THE MYELOCYTES PLOTTED IN HUNDREDS, FROM THE SUPRA-VITAL DIFFERENTIAL COUNTS OF INDIVIDUAL D

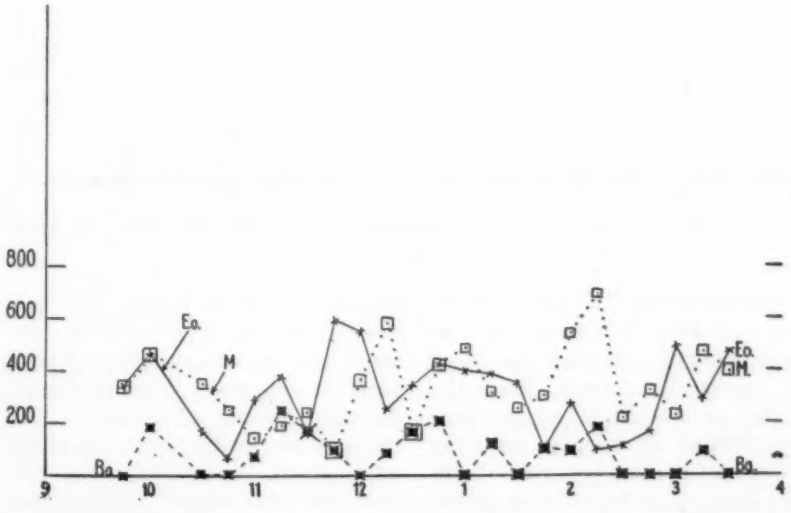


CHART 16 B. CURVES SHOWING THE EOSINOPHILIC AND BASOPHILIC LEUCOCYTES AND THE MONOCYTES PLOTTED IN HUNDREDS, FROM THE SUPRA-VITAL DIFFERENTIAL COUNTS OF INDIVIDUAL B

ings, studying the blood both in the human and in dogs. In one dog which had fasted for 24 hours there was a leucocytosis of 147 per cent. His other experiments showed a much less marked leucocytosis.

Japha (21) found no regular digestive leucocytosis in infants. He carried out experiments on himself and found that he never had any leucocytosis immediately following the morning or evening meal, the reaction first appearing 2 to 3 hours afterwards, but after the mid-day meal he had it promptly, whether this meal was poorer in albumin than the morning or evening meals or not. If he omitted his mid-day meal the leucocytosis became apparent at the usual time, *i.e.*, 3 to 6 o'clock in the afternoon. He became convinced of of the existence of daily leucocytic variations which are independent of the taking of nourishment. These periodic leucocytic variations reached their highest levels in the afternoon, decreasing again toward evening. He thought digestive processes had a certain effect in increasing these variations, but that these could only be properly estimated when considered in connection with the daily variations.

Rothacker (33) carried out investigations on the question of digestive leucocytoses on 30 persons who had received various types of food. He found that about one hour after a meal an increase was noticeable in the total number of leucocytes and this increase usually reached its maximum about three hours after the food had been taken. The increase amounted to from 800 to 3000 cells. This reaction, however, was not always present. In those cases in

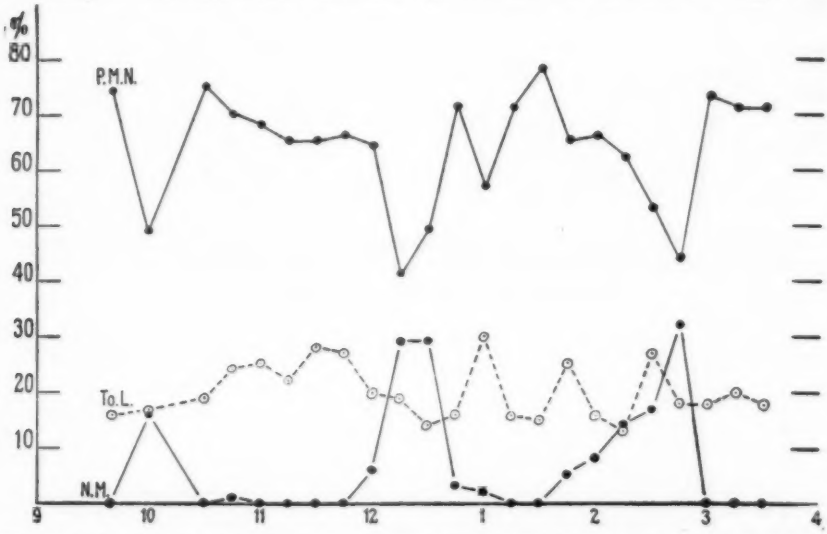


Chart 17 B. Curves Showing Data Corresponding to Those of Chart 2 B, from Individual B, Plotted in Percentages

which the reaction was noted the type of food seemed to have no effect; the leucocytosis found after the digestion of albuminous food was no more extensive than that occurring after food poor in albumin. He concludes that a digestive leucocytosis is not always a regular phenomenon, nor leucocytosis after meals rich in albumin or in fat, but only a very slight reaction after food consisting of carbohydrates alone.

Ciaccio and Pizzini (11) studied the spleen during digestion and found a hyperfunction accompanied by an enlargement of the follicles, increase

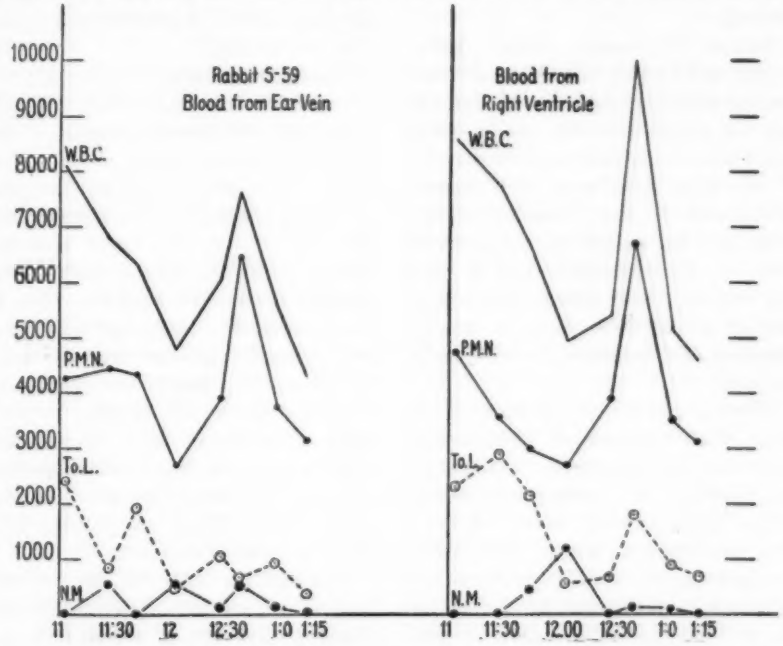


Chart 18. Rabbit S59. Curves Showing the Numbers of the White Blood-cells, the Polymorphonuclear Neutrophilic Leucocytes, the Non-motile Leucocytes and the Lymphocytes Obtained from Synchronous Supra-vital Differential Counts of the Blood of a Rabbit (Rabbit 59), Taken from the Right Heart and from an Ear Vein

is it always present in the same individual to the same degree. The lymphocytes ran a curve opposite to that of the polymorphonuclear neutrophiles during the leucocytoses in his experiments.

Sirenskij (39), in studies on the human, found a decided digestive

of megacaryocytes, and myeloid transformation of the pulp. They assume that the spleen gives a substance into the blood capable of being transformed into trypsin by trypsinogen, and which is either the product of increased leucolysis or else a secretion of the megacaryocytes.

Charteris (10) studied the blood in a case of prolonged fasting and found a mild increase in the number of white blood-cells; he states that his findings are entirely opposed to those of all the other observers who have usually described decreases in the number of leucocytes after fasting.

Pirone (31) made careful histological studies of the blood-forming organs during digestion and came to the conclusion that the reaction has three phases; 1, increased destruction of the older blood-cells; 2, increased stimulation to the formation of new cells and the migration of elements into the blood-stream; and 3, that the bone-marrow, spleen and lymph-glands all showed signs of hyperfunction and increased hematopoietic activity.

Avezzu and Pozza (2) have found that the relations of gastric-acid secretion and leucocytic reactions are demonstrated by numerous experiments and clinical cases, showing that substances which perceptibly increase gastric acidity cause a leucocytosis in proportion to the gastric effect. On the other hand, in persons having a hypo-acidity, they found a post-alimentary leucopenia. Calcium chloride, magnesium chloride and sodium chloride, given by mouth, intravenously, or intraperitoneally, cause the secretion of hydrochloric acid. They refer to the observations of other investigators who have shown that solutions of sodium bromide and sodium iodide have constantly given leucocytic reactions coincident with the increase in hydrochloric acid. They also refer to several investigations in which the administration of hydrochloric acid by mouth produced a leucocytosis wholly similar to that obtained during digestion; while there was a definite leucopenia following the administration of sodium bicarbonate. From these observations they conclude that the acid-base equilibrium in the stomach is the chief factor in producing a digestive leucocytosis.

Among the most interesting of the modern concepts is the question of specific substances capable of destroying specific tissue cells. Antibodies have been prepared for lymphocytes, blood platelets, kidney cells, liver cells, etc., and these have behaved quite in accord with those already known for bacteria. But it has been further suggested that there are normally present in the body certain lysins, which are capable of breaking down the leucocytes and hence have been called leucolysins. Such a concept has been supported strongly by many authors, among whom Manoukhine and Krolunitsky deserve especial mention.

Manoukhine (24, 25) has shown that there are two ferments in the blood-stream, one of which destroys the white blood-cells and another which neutralizes the first one. These ferments he considers as the cause of digestive as well as of other types of leucocytoses. Krolunitsky (22, 23) has come to similar conclusions and both he and Manoukhine believe that the leucolysin is derived from the spleen, while the antileucolysin is derived from the liver. Krolunitsky has also shown that excitation of the vagus causes an increase in the number of the white blood-cells.

Finally, it is important to refer

to some of the theories which have been evolved in trying to determine what are the factors causing the changes in the number of the white blood-cells, as well as those involved in maintaining these cells at a fairly constant general level. We have already pointed out that much work has been done on the rôle played by ferments in the control of the leucocytes. We have reviewed the work of a number of investigators who have considered the digestion-leucocytoses to be the direct effect of the food substances or as the result of an acid-base equilibrium; but these factors have not yet solved the problem.

Probably much importance must be also attributed to variations in blood-volume and to the question of the distribution of the cells between the various vascular beds. These factors have been discussed very widely, and it is necessary to refer both to them and to some other factors of the daily leucocytic variations.

Türk (42), in his discussion of the question of the daily variations in the leucocytic count, states that in the early morning, while the individual is still at complete rest, and before food has been taken in any appreciable quantity, the leucocyte count is at its lowest point,—in healthy human beings under 5000. This diminution is in the polymorphonuclears, the lymphocytes being at an average figure, at such a time forming 35 to 40 percent of the total number of white blood-cells. This picture becomes changed during the forenoon and essentially so during the early hours of the afternoon, when the leucocytes reach their highest point, sometimes over 10,000. During the evening the white blood-cells again decrease. These variations take place whether food has been omitted or not. Türk thinks that the taking of nourishment does play a considerable part in the degree and type of the daily variations, and he is also certain that food, especially rich in fats and albumins, causes increased using up of leucocytes and that it is also capable of acting as a productive stimulant to the myeloid and lymphoid hematopoietic centers. Furthermore, Türk thinks that the various studies of the circulating blood have revealed many facts which coincide with histological observations made on the bone-marrow, spleen and lymphatic systems, indicating that during digestion there is an increased usage of cells in the periphery and an increased productive activity of the hematopoietic tissues.

Mironesco (27) found that the leucocytes were quite unequally divided between the different circulatory beds. His results have not been confirmed by all the investigators dealing with this problem but must be taken into consideration. He found that the blood from the rabbit's ear-vein contained 14,200 white blood-cells, that from the vena cava 5300 and that from the heart 3200. He also found that in cases of scarlatina the capillary blood (as obtained from skin punctures) contained about 10,000 less cells than he found in venous blood taken from the arm-veins. These results were not always uniform but, nevertheless, he feels convinced that different parts of the vascular system con-

tain different percentages of white-blood-cells.

Schwenkenbecher and Siegel (38) made numerous counts at short intervals from various blood vessels in both rabbits and dogs and found that they never obtained the same numerical content and that often the deviation was equal to many hundreds of cells, sufficient to eliminate the possibility of technical error. They always found larger numbers of cells in peripheral than in central vessels after an ether narcosis had been commenced, but in normal animals they found approximately an equal distribution of cells.

Mauriac and Maureau (26), after giving a careful review of all the work done on the fragility of the leucocytes and on their distribution, conclude that the unequal partition of the white blood-cells between the peripheral and central vessels explains in part the variations in the leucocyte count and that it is probable that vaso-motor reactions play a part in this distribution. But they consider that they have no right to believe that this alone is responsible for all the leucocytic oscillations. Study of the fragility of the leucocytes has shown that in cases of shock this phenomenon aids in explaining many cases of leucopenia. These conclusions are based on the fact that Mauriac and Moureau found that certain pathological phenomena, *e.g.*, pneumonic crises, the crisis in hemoglobinuria, anaphylactic shock and the leucopenia following hemorrhage, are all accompanied by increased leucocytic fragility. They also find it striking that many hyperleucocytoses are accompanied by increased leucocytic resistance, and suggest that this is an indication that there is an increased activity of the leucopoetic centers and that they are furnishing these younger and more resistant cells. But in conclusion the authors say that there are doubtless still other factors involved and that the analysis of leucocytic variation presents a vast problem in modern medicine.

Thus, it is obvious that many observers have recognized the fact that there are considerable variations taking place in the numbers of blood-cells in the circulation of the normal animal as well as in diseased conditions and after experimental procedures. They have suggested a great variety of explanations as to the mechanism involved in these changes; among these being vaso-motor phenomena, variations in blood-pressure and volume, the effect of leucolysins and antileucolysins, the destructive and stimulative effects of food substances and the acid-base equilibrium resulting from the secretion of hydrochloric acid in the stomach. But it has seemed to us that all of these observations have been made with an insufficient basic knowledge of the normal variations which occur in the daily rhythms of the blood-cells, so that we have attempted, in the work which we are presenting here, to formulate a new base line upon which further investigation of the factors involved in leucocytic variations may be founded.

### RHYTHM OF THE TOTAL WHITE BLOOD-CELLS

The most striking result that we have obtained from our data is that there is an approximately hourly

rhythm in the number of the white blood-cells. This is also true of the red blood-cells, of the platelets, and of the reticulated reds. It is, however, only the rhythm of the white cells that we are reporting at this time.

In Charts 1 to 6 the curves of the total white cells, together with the curves of the active leucocytes, the total lymphocytes, and the non-motile leucocytes from the six experiments are shown. The upper line on each chart is the curve of the total white counts; the data for the other three lines were obtained by multiplying the total number of cells by the percentage of each type from the supra-vital differential counts. The hours are indicated on the abscissae, the numbers of the cells in thousands on the ordinates. It is clear that it is only the actual numbers of the cells that show the rhythms, and not the percentages, as will be seen by comparing Charts 2 B and 17 B. On Chart 17 B, it will be seen that the percentages of the neutrophilic leucocytes remain approximately steady with the exception of the times when there are marked showers of non-motile cells.

It will be noted by comparing the first six charts that there is an hourly rhythm of the white blood-cells. On Charts 1 A and 2 B this rhythm is really quite exact, there being actually a peak and a depression of the curve for each hour; on the other four charts an occasional interval of an hour and a half between the peaks makes the curves not quite so symmetrical; the curve of Chart 5 E is practically a half-hourly rhythm with the higher peaks at the hourly intervals.

The second conclusion about the curves is that without exception they all run higher in the afternoon. That this is not simply a concentration phenomenon is demonstrated by our curves of the red blood-cells, which do not show a corresponding rise (see Chart 4 D). In the curve of Chart 1 A, it will be noted that the lowest points of the curve fall between eleven and twelve o'clock; in comparing the curve of the afternoon counts with the curve of the morning counts, the most striking point is that the range of the variation between the low and high peaks is decidedly greater in the afternoon hours. During the afternoon, the low points are slightly higher and the high points decidedly higher than the corresponding levels in the morning. This subject had neither food nor drink from the time of dinner the preceding night to the end of the experiment.

The curve of Chart 2 B covers a shorter number of hours. In this chart, two equally low points of the curve fall at 10:45 and 11:15, respectively, while the higher levels extend from 11:45 on. During the afternoon hours on this curve, the lower level is markedly higher with the exception of the one count at 1:15, so that the curve of the latter part of the afternoon tends to show less fluctuation. This is the individual who drank a glass of water at 12:15 and nearly a pint of cream at one o'clock, but otherwise had neither food nor drink. Our record shows that fat appeared in the blood at 2:30, and was abundant at 2:45, showing that the experiment was long enough to include some digestion.

The curve of Chart 3 C illustrates

again the marked fluctuations in the number of cells during the early hours of the afternoon, while after two o'clock the lower level of the curve is markedly raised. This individual had neither food nor drink during the entire period. On Chart 4 D, the high peak at 1:15 is quite out of alignment with all of the other curves and this was the one time in the whole series in which the curve is so markedly high during the morning hours. We have no explanation for this variation at the present time. With that exception, this curve represents the general rule of a lower curve and less fluctuation in the morning hours; during the afternoon, the fluctuations are marked, the curve being like that in Chart 1 A. This individual had a lunch rich in proteid at 12 o'clock. We have added to Chart 4 D the curve of the red blood-cells, plotted in millions, to show that there is no rise in the red blood-cells in the afternoon.

The curve of Chart 5 E shows practically a half-hourly rhythm. On this curve the fluctuations are rather greater in the morning, while the entire curve of the afternoon is set at a higher level, with only two marked fluctuations. There is a marked low level between 12 and 1 o'clock. In general, the curve of Chart 6 F is strikingly like that of Chart 5 E. This curve does not show an exact hourly rhythm, since there is one interval of an hour and a half during the morning, but it shows the same high average level for the afternoon. The person whose blood-cells are represented on Chart 5 E had neither food nor drink, while the one on Chart 6 F had a pint of milk for breakfast, taken at 8:15 a.m.

When all of these curves of the total white cells are reviewed, it will be seen that they all agree in the fact that the curves of the white cells during the afternoon hours are consistently higher than during the morning. When the curves of the afternoon hours are compared, it will be seen that they fall into three types; first, the type shown on Charts 1 A and 4 D, in which the fluctuations during the afternoon are very marked, with only a slight raising of the lower levels; second, the type represented on Charts 5 E and 6 F, in which there is a marked raising of the mean level and little fluctuation; third, the type shown on Charts 2 B and 3 C, in which the curves of the afternoon start with sharp fluctuations and end with a marked raising of the mean level, that is to say, a delay in the rise of the mean level. There is some tendency to have a very low level, though not always the lowest level, between the hours of 12 and 1:30 p.m.

The normal limits of variation in the number of the white blood-cells have long been established as being from 5000 to 10,000 cells per cubic millimeter, and very many recent experiments on experimentally induced leucopenias in the literature indicate that there are marked daily variations in the number of white cells; but we know of no previous experiments which clearly demonstrate that the entire normal range of variation in the number of white cells is covered rhythmically each day. Our series of six human counts is of course too small to allow such a deduction, but, taken in conjunction with our many series on the blood of the rabbit, which all demonstrate

the same thing, we feel justified in the conclusion that the whole range of the number normal in an animal is covered each day.

The curves shown on our charts indicate that there are considerable variations in each individual with regard to both the high and the low limits of the counts. The limits which we have observed are given in Table 1.

It is clear from this table that Chart 1 A represents a consistently high average, while Charts 5 E and 6 F both represent a low average.

TABLE 1

*Total number of white blood-cells*

| | LOW | TIME | HIGH | TIME | NUMBER OF COUNTS | NUMBER OF CELLS COUNTED |
|---|---|---|---|---|---|---|
| Chart 1 A | 7,200 | 11:15 | 13,680 | 1:30 | 29 | 2,900 |
| Chart 2 B | 6,280 | 10:45, 11:15 | 10,920 | 2:30 | 24 | 2,400 |
| Chart 3 C | 5,560 | 1:30 | 10,400 | 2:30 | 30 | 3,000 |
| Chart 4 D | 5,160 | 2:00 | 11,640<br>10,400 | 11:15<br>4:45 | 33 | 3,300 |
| Chart 5 E | 5,600 | 10:30 | 9,920 | 4:15 | 30 | 3,000 |
| Chart 6 F | 4,200 | 12:15 | 8,700 | 3:00 | 29 | 5,650 |

In a general way, it will be noted that the high limits tend to be about twice the low levels.

From these studies of the curves of the total white counts we then conclude that the entire normal limits of the variation in the number of cells is covered in the period of eight hours each day, that the highest level is about twice the lowest, that the fluctuations are rhythmical, with a marked tendency to an increase each hour, and that the curve in the afternoon is markedly higher than in the morning hours, sometimes through greater fluctuations, sometimes by having a higher mean level. It would be of great interest to have a longer experiment, to know the type of curve for the night, but a longer experiment is beyond the endurance of a limited number of workers. It would also be interesting to repeat the experiment on the same subject, in order to know if the same individual characteristics of the curve could be repeated. This has not yet been done. From the results which we have obtained on the two individuals who had food (Charts 2 B and 4 D), we suggest that any experiments made to establish the leucocytosis of digestion should be made on the basis of the known normal curve. If digestion leucocytoses are to be tested on individuals who are ill, it would be necessary to have some data as to whether the normal rhythm is itself altered in the disease from which they are suffering.

## RHYTHM OF THE MOTILE AND NON-MOTILE NEUTROPHILIC LEUCOCYTES

When we turn from the curves of the total number of the white blood-cells in a cubic millimeter of blood at a given moment to the analysis of the variation of the different types of

cells from which this rhythm is compounded, we have one of the most fundamental of the problems of hematology, namely, what are the different stimuli that cause the development of these types of cells and how are they called into the circulation? We believe that one of the necessary factors in the analysis of this problem is a knowledge of the characteristic rhythms of these cells.

In including the non-motile cells in the supra-vital differential counts to obtain the percentages from which our curves are plotted, we have assumed that the non-motile forms were included in the total white counts. We decided to include the non-motile cells because we had tested the reaction of the non-motile cell of our supra-vital preparations by running Türk's solution under the coverslip while we watched the cell. In these experiments, the non-motile cell was not damaged in the least. When, however, we ran 33 per cent alcohol under the coverslip, the granules dissolved. This observation, that the non-motile cell is not damaged by the Türk's solution, does not prove that it may not be damaged by the shaking of the pipette necessary for the adequate mixing of the blood and diluting solution. Moreover, it is not at all possible to discriminate the non-motile cells in the counting chamber; nevertheless, we think that the correspondence of the curves plotted from the supra-vital and from the fixed films, compared with the curves of the total white counts, indicate that we are justified in concluding that the non-motile cells appear in the total white counts. There may, of course, be some disintegration of non-motile cells in the shaking of the pipettes, but in our judgment by no means so many non-motile forms are destroyed in the pipette as to vitiate our general type of curve. Furthermore, even if the non-motile cells are broken down in shaking, the resulting free nuclei would be included in the total count. We have found, as a matter of technique, that if the Türk's solution is centrifuged for a half hour before an experiment there is very little debris to be found in normal blood to vitiate a count. Even if there is some disintegration of dying cells in the pipettes, it would not in any sense vitiate the curves of the total white count as showing an hourly rhythm of the leucocytes, but it might possibly alter the curves of different types to some slight extent. This, however, does not seem very probable to us.

By examining the different curves shown in Charts 1 to 6 it is clear that it is the motile leucocytes that most consistently follow the curve of the total white cells. For example, the the consistently higher range in the number of white blood-cells in the afternoon, as contrasted with the range of the morning hours, is characteristic also of the motile leucocytes. This correlation with the curve of the total number of the cells may, of course, be associated wholly with the fact that the greater percentage of the neutrophilic forms stamp their rhythm on the total white count. Here it is also to be noted that the special types of the curve of the total cells in the afternoon, *i.e.*, the type with marked fluctuations, the type with a high mean level, or the mixed type, are quite accurately followed by the motile leucocytes.

However, though the correspon-

dence between the curve of the motile leucocytes and that of the total white count is marked, nevertheless, a careful study of the two curves will show that the general correspondence is interfered with by showers of non-motile cells. If each chart is followed, it will be seen that the curves of the non-motile cells are quite irregular, but in each one there are two or three very marked showers of the non-motile cells, while for the rest of the time there are either few or none of these cells. In a general way all the curves show that the marked peaks on the curves of the non-motile cells tend to be opposite a depression of the curve of the motile forms. Indeed, this reciprocal relation is not only the rule for the marked showers but for the lesser ones as well. This must be true, since the non-motile forms are dying neutrophilic leucocytes. Of this we are entirely sure, because we have repeatedly seen all the transitions between these forms and the motile leucocytes. For example, in a preparation in which there are many non-motile forms, we have repeatedly found a few leucocytes that still react to the stain but have rounded up and stopped moving. Otherwise, every leucocyte in the vital preparations is in constant motion.

While it is a general rule that the showers of non-motile cells correspond to depressions in the curve of the motile cells, there are a few exceptions. For example, on Chart 3 C, at 11:30, and on Chart 5 E, at 1:15, there are examples of a condition when the peak of the curve of the non-motile cells is not at the lowest point on the curve of the motile cells but at a point at which the curve of the motile cells is rising. There are several places where this is true. In our judgment, this means that we have not struck the exact moment in our experiment at which the shower of the non-motile cells was at its maximum, and it would not be reasonable to expect that the arbitrary interval of 15 minutes would uniformly give an exact correlation between the maximum peak of the curve of the non-motile cells and the minimum of the curve of the motile leucocytes. The frequency with which we have the close correspondence seems to us to be quite remarkable, in view of the great number of factors which obviously have a part in regulating and controlling the numbers of cells in the circulating blood. Chart 2 B is especially interesting in connection with the showers of the non-motile cells, for there were three prolonged showers of the non-motile cells and almost none at any other time. In this instance the higher peak of the first shower falls opposite a rising curve on the motile leucocytes, but the curve of the motile leucocytes continues to rise markedly while the non-motile forms drop almost to zero. We have studied the curves to see if we could discover any correlation between the showers of the non-motile cells and the increase in the motile leucocytes. In general, in all of the curves there is a rise in the motile leucocytes, following the showers of the non-motile cells, after a period of 15 to 30 minutes. This occurs in almost every instance. The amount of the rise cannot be said to be proportional to the showers of the non-motile

cells but it is certainly striking how constant is the occurrence. The converse is not true, for there are several cases in which a marked rise in the curve of the motile leucocytes was not immediately preceded by a shower of non-motile cells.

If we consider the rises of the curve of the leucocytes after the showers of dead cells as a replacement of the dead cells, it is so remarkable that it should take place as quickly as 15 minutes that we have been very loth to draw such a conclusion. When the curves are studied carefully, it will also be noted that there is another possible condition with regard to the curve of the leucocytes following the showers of non-motile cells; this is, that each shower of non-motiles is followed by a rise in the motile leucocytes after an interval of about two hours, and we have no evidence that would lead us to discriminate as to which rise in the leucocytes, the immediate or the delayed, is actually to be correlated with a given shower of the non-motile cells. There is no constant proportional relationship to guide us in this regard. If, however, the immediate response be an actual replacement of the dead leucocytes, then we could only imagine that it was to be correlated with a vasomotor phenomenon in which the leucocytes were to be regarded as already within the circulation, perhaps in an extramedullary region or perhaps within the sinuses of the bone-marrow and, therefore, ready to be swept into the circulation in response to a vasomotor change produced by the sudden liberation of some substance into the circulation by the great mass of dying leucocytes. From the known rate of movement of leucocytes, we could not imagine them actually entering the sinuses from the intersinusoidal spaces in the bone-marrow in the interval of time which occurs between the showers of non-motiles and the immediate subsequent rise in neutrophilic granulocytes. In this connection Barcroft's recent studies (4) on the spleen as such an extramedullary source of blood-cells are most suggestive. In our studies we are inclined to include also the capillaries of the viscera in connection with this phenomenon. In Table 2 we are showing the number of times in which the non-motile cells appear in the vital counts. From this table it appears that they are present rather more than half the time; but from the charts it is evident that the majority of the showers are of small proportions, with an occasional very marked shower averaging about 30 per cent.

TABLE 2

| | | NON-MOTILES WERE PRESENT | NON-MOTILES WERE ABSENT |
|---|---|---|---|
| Chart 1 A | 29 counts | 18 (1 to 25%) | 11 counts |
| Chart 2 B | 24 counts | 12 (1 to 32%) | 12 counts |
| Chart 3 C | 30 counts | 26 (1 to 27%) | 4 counts |
| Chart 4 D | 33 counts | 22 (1 to 32%) | 11 counts |
| Chart 5 E | 30 counts | 18 (1 to 31%) | 12 counts |
| Chart 6 F | 29 counts | 25 (0.5 to 36%) | 4 counts |

We have sought to analyze the non-motile showers with reference to the constant afternoon rise of the leucocytes. In a general way there is a marked shower or showers of non-motile leucocytes in each curve between the hours of 12 to 1:30, but these showers cannot be regarded as proportional to the afternoon rise in the motile forms. We then counted the number of non-motile cells appearing from the beginning of the experiment up to one o'clock and the number from one o'clock to the end of the experiment. These data are shown in Table 3. It will be seen from this table that the number of the non-motile cells during the morning was consistently higher, but not markedly so, the excess being only about a thousand cells. It is probable that these figures cannot be correctly compared, because the actual length of time of all the experiments was not identical, so that the number of cells counted varied; at any rate we are unable to show that the number of dying cells during the morning hours is sufficiently greater than in the afternoon to account for the rise in the leucocytes in the afternoon. However, this would not invalidate the suggestion that the dying cells were concerned in the maintenance of the normal level of the white blood-cells. Among many others, the factor of the changes in the acid-base equilibrium of the plasma must ultimately be considered in this analysis. And at present we have no data on this phase which can be correlated with our other findings.

We are giving in Tables 4 and 5 the percentages of the cells from the supravital and the fixed films for the six series. As can be seen from Table 4, the total percentage of non-motile cells for the entire day is not high, averaging 6 per cent in the supravital films from 19,750 cells counted. But when it is considered that a given shower of the non-motile cells may reach the astonishing figures of from 25 to 36 per cent of the total number of the white cells (Table 2), or approximately from a third to nearly a half of the number of the neutrophilic leucocytes in a drop of blood, it is clear that any such periodic death rate of the leucocytes constitutes a factor which must be considered in making blood counts. We regard our figures as only crude estimates of the numbers of these cells; the showers are usually transient; we believe that the distribution of these cells in the blood throughout the vascular system is probably irregular, owing to their tendency to clumping, and that perhaps this irregularity is great enough to explain quite adequately why we cannot make a definite correlation between such phenomena as the rise of the leucocytes in the afternoon and the showers of the non-motile cells. In order to arrive at any adequate

TABLE 3

| | NON-MOTILE CELLS FROM 9 A.M. TO 1 P.M. | NON-MOTILE CELLS FROM 1 P.M. TO 5 P.M. |
|---|---|---|
| Chart 1 A | 7,331 | 5,370 |
| Chart 2 B | 8,150 | 7,046 |
| Chart 3 C | 9,296 | 6,973 |
| Chart 4 D | 8,890 | 8,193 |
| Chart 5 E | 6,184 | 4,786 |
| Chart 6 F | 6,195 | 4,920 |
| Average | 7,624 | 6,211 |

analysis of the extent and significance of these showers that we have observed in the peripheral blood, it will be essential to ascertain whether such showers also occur in other parts of the circulation.

In the teaching of making blood counts and in clinical practice, it is a common experience that occasional blood counts are made which are quite out of alignment with the average blood in the normal and in disease. Such discrepancies have been accounted for on the basis of errors in technique, often when it was impossible to determine what the errors have been. If there is any such periodic death rate as our

TABLE 4

*Average percentage from supra-vital films*

| CHART | NEUTROPHILIC LEUCOCYTES | NON-MOTILE LEUCOCYTES | TOTAL NEUTROPHILIC LEUCOCYTES | BASOPHILIC LEUCOCYTES | EOSINOPHILIC LEUCOCYTES | SMALL LYMPHOCYTES | INTERMEDIATE LYMPHOCYTES | LARGE LYMPHOCYTES | TOTAL LYMPHOCYTES | MONOCYTES | MYELOCYTES | DAMAGED CELLS | AVERAGE PERCENTAGE | NUMBER OF CELLS COUNTED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 A | 63.34 | 4.58 | 67.92 | 0.89 | 1.78 | 6.86 | 8.84 | 5.95 | 21.89 | 6.50 | 0.10 | 0.60 | 99.44 | 2,400 |
| 2 B | 64.08 | 6.74 | 70.82 | 1.00 | 3.86 | 8.58 | 8.12 | 3.04 | 19.91 | 4.08 | 0 | 0 | 99.50 | 2,400 |
| 3 C | 63.74 | 7.03 | 70.77 | 1.23 | 1.96 | 6.71 | 10.57 | 2.75 | 20.03 | 5.56 | 0 | 0.23 | 99.78 | 3,000 |
| 4 D | 68.85 | 7.18 | 76.03 | 0.96 | 3.60 | 6.27 | 6.42 | 1.51 | 14.20 | 4.57 | 0.30 | 0.24 | 99.90 | 3,300 |
| 5 E | 58.13 | 4.90 | 63.03 | 1.00 | 6.40 | 9.43 | 11.90 | 1.20 | 22.53 | 7.00 | 0.03 | 0 | 99.99 | 3,000 |
| 6 F | 55.62 | 6.17 | 61.79 | 2.22 | 9.06 | 11.31 | 8.63 | 0.32 | 20.13 | 6.43 | 0.05 | 0.13 | 99.94 | 5,650 |
| Average | 62.29 | 6.10 | 68.39 | 1.22 | 4.44 | 8.19 | 9.08 | 2.44 | 19.73 | 5.69 | 0.08 | 0.20 | 99.75 | 19,750 |
| | | | | | | | | | | | | | 99.73 | |

TABLE 5

*Average percentages from fixed films*

| CHART | NEUTROPHILIC LEUCOCYTES | NON-MOTILE LEUCOCYTES | TOTAL NEUTROPHILIC LEUCOCYTES | BASOPHILIC LEUCOCYTES | EOSINOPHILIC LEUCOCYTES | SMALL LYMPHOCYTES | INTERMEDIATE LYMPHOCYTES | LARGE LYMPHOCYTES | TOTAL LYMPHOCYTES | MONOCYTES | MYELOCYTES | DAMAGED CELLS UNCLASSIFIED | AVERAGE PERCENTAGE | NUMBER OF CELLS COUNTED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 A | 53.90 | 7.65 | 61.55 | 0.459 | 0.838 | 14.00 | 13.548 | 0.827 | 28.38 | 8.40 | 0.043 | 0.31 | 99.94 | 11,000 |
| 2 B | 57.77 | 9.21 | 66.98 | 0.46 | 1.83 | 16.79 | 6.45 | 0.15 | 23.39 | 7.24 | 0.02 | 0 | 99.92 | 4,000 |
| 3 C | 52.75 | 15.77 | 68.52 | 0.99 | 0.97 | 9.03 | 12.36 | 0.62 | 22.01 | 6.57 | 0 | 0.07 | 99.13 | 8,000 |
| 4 D | 61.03 | 9.83 | 70.86 | 0.53 | 1.85 | 6.56 | 11.83 | 1.06 | 19.45 | 6.31 | 0.23 | 0.02 | 99.25 | 11,400 |
| 5 E | 58.22 | 7.47 | 65.69 | 0.96 | 2.87 | 7.84 | 13.40 | 1.32 | 22.57 | 7.19 | 0.04 | 0.64 | 99.95 | 11,150 |
| 6 F | 52.20 | 10.45 | 62.65 | 0.89 | 5.59 | 13.02 | 10.56 | 0.41 | 23.99 | 6.56 | 0.017 | 0.069 | 99.76 | 12,200 |
| Average | 55.97 | 10.03 | 66.04 | 0.71 | 2.32 | 11.20 | 11.35 | 0.73 | 23.31 | 7.04 | 0.05 | 0.20 | 99.65 | 57,750 |
| | | | | | | | | | | | | | 99.60 | |

figures show, we have here a factor which it will be necessary to consider in analyzing such discrepancies.

### CORRELATION OF THE CURVES FROM THE SUPRA-VITAL STUDIES WITH THOSE FROM FIXED FILMS

From our early studies of the non-motile cells we came to the conclusion that the non-motile forms appeared in fixed films as free nuclei because the alcohol of the fixative dissolved the granules in the non-motile phase. All of our further studies have confirmed this view, but we have found, in addition, that the non-motile cells of the supra-vital preparations appeared in the fixed films in several other forms, corresponding to different stages in the cycle of degeneration from the normal cell to the free nucleus, thus making the complete analysis of the dying cells a very complex matter. We are entirely convinced that such an analysis was impossible without the clear and definite results obtained with the supra-vital technique.

We made the films for the fixed preparations by pulling coverslips apart, using but two coverslips for each interval. We stained the preparations on both coverslips and found that it was necessary to count both preparations and take an average of the two in order to get a correlation between the supra-vital counts and the counts of the fixed films. We have found that the counts of the two different coverslips are quite variable. In general, when most of the blood is on one coverslip, that one tends to correlate with the supra-vital count, but when the blood is equally divided, each coverslip may correlate, or neither one may do so, and the difference is often 10 per cent or more. Therefore, we counted both coverslips, usually 200 cells on each, and took the average. Thus we are quite confident that a count which is an average of both coverslips from a single drop of blood is more apt to be representative of the original drop. This seems to us quite logical, because there is no reason why the different types of white cells should be distributed between the two coverslips in the same proportions that obtain in the blood-stream. In general, the number of cells counted in the supra-vital counts was about 3000 and for the corresponding differentials on fixed films the number ranged from 4000 to 12,000. For the experiment shown on Chart 7 F, we took two pairs of coverslips, counted 200 cells from each pair, and averaged the four counts.

In making the differential studies from the fixed films we found it necessary to count the leucocytes under four headings; first, the intact normal cells; second, a group which we have called "fragile" cells, in which the membrane had been ruptured and the full content of well-stained granules was scattered near by; third, a group of free nuclei with some poorly stained granules or with no granules in the neighborhood; and fourth, certain pale cells in which the cellular membrane had not ruptured but in which all the granules seemed to have been dissolved out, leaving a very pale, structureless cytoplasm. After a careful study of our records we have grouped all of the last three types together to correspond with

the non-motile cells of the supra-vital counts. This we have done because in this way we obtained a better correlation between the percentage of the non-motile cells in the two types of counts, supra-vital and fixed, as is shown in Table 6. For example, the series represented by the first row on Table 6, corresponding to the data shown in Chart 1 A, the combined group of fragile cells, pale cells and free nuclei, had a range of variation of from 1 to 30 per cent to be compared with from 0 to 25 per cent of the non-motile cells from supra-vital films, while the range of variation of the free nuclei alone was from 1 to 10 per cent.

TABLE 6

*Range of variation in percentages from supra-vital and fixed films*

| CHART | | NEUTROPHILIC LEUCOCYTES | NON-MOTILE LEUCOCYTES | BASOPHILIC LEUCOCYTES | EOSINOPHILIC LEUCOCYTES | SMALL LYMPHOCYTES | INTERMEDIATE LYMPHOCYTES | LARGE LYMPHOCYTES | TOTAL LYMPHOCYTES | MONOCYTES |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 A | Supra-vital | 34–75 | 0–25 | 0–6 | 0– 6 | 0 –17 | 2–19 | 0–26 | 8–37 | 3–15 |
| | Fixed | 37–60 | 1–30 | 0–1.75 | 0– 1.75 | 3 –40 | 5–23 | 0– 4.3 | 19–45 | 2–12 |
| 2 B | Supra-vital | 41–78 | 0–32 | 0–4 | 0–10 | 0 –20 | 1–16 | 0–10 | 12–30 | 1– 9 |
| | Fixed | 49–68 | 3–19 | 0–1.75 | 0– 4 | 2 –32 | 6–18 | 0– 2 | 16–44 | 3– 8 |
| 3 C | Supra-vital | 53–78 | 0–27 | 0–6 | 0– 4 | 0 –16 | 4–19 | 0–10 | 13–32 | 2–10 |
| | Fixed | 40–67 | 2–34 | 0–2 | 0– 3 | 3 –23 | 3–24 | 0– 1.75 | 13–35 | 2– 9 |
| 4 D | Supra-vital | 50–85 | 0–32 | 0–5 | 0– 9 | 0 –13 | 1–15 | 0– 9 | 6–24 | 0–10 |
| | Fixed | 47–75 | 1–31 | 0–1.3 | 1– 5.5 | 0.5–12 | 5–16 | 0– 4.25 | 11–26 | 2–10 |
| 5 E | Supra-vital | 31–73 | 0–31 | 0–3 | 3–11 | 2 –18 | 5–23 | 0– 7.7 | 12–34 | 0–13 |
| | Fixed | 39–76 | 1–29 | 0–3 | 1– 5.4 | 1 –16 | 1–28 | 0– 4 | 12–33 | 4–12 |
| 6 F | Supra-vital | 27–68 | 0–36 | 0–6 | 4–14 | 3 –20 | 3–20 | 0– 1.5 | 13–32 | 0–12 |
| | Fixed | 40–63 | 3–32 | 0–2 | 3– 7.75 | 7 –23 | 6–15 | 0– 1.6 | 13–33 | 4–10 |
| Average | Supra-vital | 39–76 | 0–30 | 0–5 | 1– 9 | 0.8–17 | 2–18 | 1–12 | 10–31 | 1–10 |
| | Fixed | 40–66 | 0–28 | 0–1.6 | 0– 4.56 | 2.7–24 | 4–21 | 0– 2.9 | 14–36 | 2–10 |

TABLE 7

*Range of variation in total numbers, supra-vital films*

| CHART | NEUTROPHILIC LEUCOCYTES | NON-MOTILE LEUCOCYTES | BASOPHILIC LEUCOCYTES | EOSINOPHILIC LEUCOCYTES | TOTAL LYMPHOCYTES | MONOCYTES |
|---|---|---|---|---|---|---|
| 1 A | 3,672–9,165 | 0–2,236 | 0–456 | 0–276 | 672–3,774 | 216–1,710 |
| 2 B | 3,292–7,448 | 0–2,636 | 0–251 | 62–585 | 1,024–2,948 | 97– 691 |
| 3 C | 3,002–7,312 | 0–1,616 | 0–432 | 0–430 | 722–2,396 | 195– 748 |
| 4 D | 2,882–8,846 | 0–2,252 | 0–424 | 0–601 | 616–2,095 | 0– 710 |
| 5 E | 2,442–6,745 | 0–1,764 | 0–236 | 172–882 | 792–2,758 | 0–1,274 |
| 6 F | 1,695–5,711 | 0–2,346 | 0–324 | 239–864 | 712–1,852 | 0– 754 |

In regard to the fragile cells, they are certainly the group most likely to be considered as having been damaged by the technique. In the supra-vital technique we have found very few cells in which the membrane had ruptured; occasionally, in the fixed films, these cells are numerous. Since we think that they belong with the non-motile cells of the supra-vital films, it is possible that they represent the cells just about to pass into the non-motile phase, the ones that have rounded up in the supra-vital films but still show granules that stain in neutral red; for this, of course, we have no conclusive evidence, and it is entirely possible that some of them are made by a too rough handling of intact cells. With regard to the pale cells, in which the granules have entirely disappeared from the cytoplasm, and the nuclei with a few faintly stained granules or with no granules at all near them, we feel more confidence. We consider that they are the non-motile leucocytes from which the granules happen to have been dissolved completely by the alcohol of the fixative; the only difficulty which comes up in connection with such an interpretation of these cells is due to the fact that the nucleus of the non-motile cell has often become so edematous as to appear round; such a pale cell with a round nucleus might then be taken for a lymphocyte. In well stained preparations we do not think this difficulty great, but if the film of blood has stood for some time before being stained, the difficulty is greatly increased.

In the group of free nuclei, we included all structures which we were sure had been nuclei. This group includes every transition between the cells which we have called the fragile leucocytes, with intact, well stained granules on the one hand, and, on the other hand, the formless masses of debris which we can neither interpret in the supra-vital technique nor in fixed films. In the group of free nuclei we have included nothing in which there was not clear evidence of nuclear structure. Perhaps the majority of these nuclei still show evidence of polymorphism, but some of them have become so edematous as to show almost no or actually no lobes.

In the supra-vital films we have usually noticed a tendency for the non-motile cells to be clumped; they are not as evenly distributed on the slides as are all of the living types; they also tend to be in the thinner areas and somewhat toward the edges of the films. This unevenness in distribution of the free nuclei is still more striking in fixed films. We have found in fixed films that both the free nuclei and the monocytes, that is to say, the largest cells—for we think that the free nuclei come from the swollen non-motile cells—have a marked tendency to be nearer the edges of the films. Thus, if one counts only the evenly spread center of a fixed film, the count will tend to be low both in monocytes and in free nuclei. In any fixed film of blood in which there are marked numbers of the free nuclei, it will be noted that, while the center of the films may be quite evenly and perfectly spread, there will be a periphery in which the red cells are splotched, leaving round areas or holes

free from red cells, but often containing a free nucleus, as if the coverslip had been greasy. It is a possibility that the granules of the non-motile phase, which are so highly refractive, may be in a state from which a little fat is set free. This might account for their increased refractivity and for the blotchy appearance of films of blood in the zones containing large numbers of these cells. Thus, an appearance which has been set down as an error in technique, might be due to the actual nature of the particular drop of blood itself rather than to a faulty technique in the preliminary cleaning of the glass. However, it is obvious that such an interpretation does not call for any less scrupulous care in the cleaning of slides and coverslips. With the fixed films we have systematically counted from edge to edge of the film and have tried for 200 consecutive cells.

In both the supra-vital and the fixed preparations there are occasional damaged basophilic and eosinophilic cells. Occasionally, we have seen a non-motile cell in the supra-vital preparations with granules so large that we thought that it might have come from an eosinophile. In the fixed films, the fragile eosinophiles are readily recognized. They make an exceedingly small percentage. There is an occasional free nucleus which seems to us to have come from a monocyte; these nuclei are definitely bean-shaped and stain more intensely than the free nuclei which we have identified as having come from the neutrophilic leucocytes. These are also rare, far under 1 per cent. The occasional damaged eosinophiles, basophiles and monocytes have been included with the intact cells of the corresponding types. In the supra-vital counts and in the fixed films we have found occasional small, round, damaged cells or nuclei which we have interpreted as dead lymphocytes; in the supra-vital preparations these cells may have granules which are more refractive than mitochondria; these cells we have classified separately as damaged cells, and it will be seen in Tables 5 and 6 that in both methods they run under 1 per cent. In cases of lymphatic leukemia, one may find very great numbers of degenerating lymphocytes and we think that in them there is not so marked a distinction between the nucleus and the cytoplasm as in the case of the non-motile leucocytes.

In the living preparations and in the fixed films there were many masses of debris which we were entirely unable to analyze. In the living preparations these appeared as formless masses; and it has so far been impossible to determine whether they are the last stages of the non-motile cells, disintegrating masses of platelets, or perhaps some other type of tissue debris. In the fixed films we have identified these structures as the large pink smudges which, from their streaked appearance, have frequently been considered to be collections of fibrin. We have not counted these structures in either the supra-vital or fixed preparations, because we have so far been entirely unable to determine their nature or their source.

We are giving two charts (7 F and 8 B) to show the comparison between the differentials from supra-vital and fixed films. Chart 7 F

corresponds with Chart 6 F, Chart 8 B with Chart 2 B. As in all of the studies, we think that the curves of the motile leucocytes correspond as closely as one could expect from two different drops of blood. The general points which we have already brought out in connection with the curves from the supra-vital counts, namely, the lower levels of the morning hours, the afternoon rise, and the type of the curve in the afternoon, are all the same. When we compare the curves of the non-motile cells, Chart 8 B gives the best correlation. It will be seen, on Chart 2 B, that there were three distinct showers of the non-motile cells and these are duplicated on Chart 8 B. There are, however, on Chart 8 B, more damaged cells in the intervals between the marked showers than on Chart 2 B. The correlation is fair between Charts 6 F and 7 F. We think that there are many factors which would lead us to expect that the correlation would not be more exact than our curves show. The first of these is that the non-motile cells tend to clump to such a marked degree. For this reason, we think it quite likely that the numbers of these cells in two drops of blood, even though taken consecutively, might differ markedly. That this is actually the case we are convinced, because we have several times found large numbers of these non-motile cells agglutinated about a central core of platelets.

We have made some studies in the correlation of the number of leucocytes in the blood taken from the heart and from the peripheral veins of the rabbit. These experiments are still incomplete and we do not feel as yet that we can draw any final conclusions from this work, but it seemed to us interesting and suggestive that there should be as close a correlation in the types of cells as has been generally the case. In Chart 18, we are giving an example in which the correlation was rather close between the blood taken from the two sources. It will be noticed that the non-motile shower in the heart's blood is more pronounced than that in the peripheral blood; this we assume to be, in all probability, due to the fact that the production of these cells was slightly earlier in the deep blood and had not reached its maximum in the peripheral capillaries. These experiments demonstrate conclusively that the showers of non-motiles are of wide and general occurrence and are in no sense limited to the peripheral blood. It must, however, be noted that there may not be as much variation between the blood of the right heart and that of the peripheral veins as there is between the mixed venous and capillary blood (which we certainly obtain in taking blood from the human finger) and the blood of the deep vessels. While we feel quite confident that these showers of non-motile or dying cells represent a most important factor in the physiology of the blood, nevertheless, it must be noted that the great irregularity of their occurrence indicates that the numbers of the viable cells in the blood are not so constant as has been concluded from previous work. We are thus led to conclude that the supra-vital technique gives a much more accurate picture of the actual numbers of dying cells in a drop of blood than one can

obtain by any other method so far developed. It has become wholly evident that, with the fixation and staining of these cells, we have them appearing in different ways; as fragile cells with intact granules, which may correspond to the stage in the living preparation when the cell has stopped moving but in which the granules still react to neutral red; as the clear pale cells from which all the granules have been dissolved by the alcohol; or as the free nuclei with few or no granules remaining. From the comparison of some of the curves from the fixed films with those from the supra-vital films, it was suggested to us that some of the nuclei set free from the non-motile cells in the actual circulation may remain in the blood for some little time after the cytoplasm has disintegrated. With this suggestion in mind we are now counting all free nuclei to be found in the living preparations, and hope to be able eventually to determine the rate of destruction of the cells, the time required for the degeneration of the fragments, and their eventual and ultimate fate.

### RHYTHM OF THE LYMPHOCYTES

When the curves of the total lymphocytes, as seen in Charts 1 to 6, are compared with the curves of the leucocytes, the characteristic difference is that the lymphocytes tend to have a half-hourly rhythm rather than an hourly one. That is to say, there are no marked accentuations of every fourth count in our series, but rather the curves tend to go alternately up and down at approximately each count with an interval of 15 minutes. This is evident on all of the charts, and the point emphasizes the idea we have already discussed that it is the curve of the neutrophilic leucocytes that corresponds to the total number of white cells rather than the curve of the quicker rhythm of the lymphocytes. This is probably due to the greater number of the leucocytes, the average of the limits being from 2830 to 7537, while the average limits of the lymphocytes are from 756 to 2637 cells per cubic millimeter. When the curves of the total lymphocytes are compared with each other, there is no especial characteristic common to all except this fact of the short rhythm. For example, there is no constant rise of lymphocytes in the afternoon. In Chart 1 A, there are two very marked peaks of the lymphocytes at 11:45 and at 12:45, reaching the astonishing number of 4000 cells; such a rise does not appear in any of the other curves. After these two peaks, the curve of the afternoon is only slightly higher than that of the early morning. On Charts 2 B, 3 C and 5 E, the lymphocytes are very slightly higher in the afternoon than in the morning. On Chart 6 F there is the least variation and hardly any difference between the afternoon and morning, while on Chart 4 D, from the individual who took a lunch of proteid, the curve of the lymphocytes is lower in the afternoon than in the morning. We thus find that the common characteristic of these curves of the total number of the lymphocytes is that they all tend to have a quicker rhythm than the total white cells or the polymorphonuclear leucocytes.

In the living preparations we

counted the lymphocytes under three headings: the small, the intermediate and the large cells. Such a grouping is shown in Chart 9 F. This chart gives the groups of the lymphocytes plotted on the same scale as that used for the leucocytes in Charts 1 to 6. Concerning the small cells, there is practically no disagreement between different individuals making the counts. The distinction between the intermediate and the large cells, on the other hand, brings in a personal equation as to where the line is to be drawn between these two types. In the count shown on Chart 9 F, we consistently restricted the large lymphocytes to the small group of the very largest of the lymphocytes; in some of the other counts the line was not so sharply drawn. To so restrict the group of the large lymphocytes is the more usual convention. In studying the supra-vital preparations, we have recorded whether the cells were quiescent or in motion. In general, at the temperature at which we study the preparations, namely, at about 37°, we have found that only a small proportion of the lymphocytes are actually moving, and that, of this small group, almost all are of intermediate size. However, especially in our experimental animals, in which we have to some extent stimulated the lymphocytes, we find that, in addition, both the small and the large lymphocytes are capable of movement. When the temperature of the box is 40°, many more lymphocytes move than at 37°. We have seen far more intermediate lymphocytes in motion than any other type, and the large type is the one least often seen in motion. The ability of a cell to move, we consider, in general precludes the idea that this cell is senile, so that, in our opinion, no one of the three groups of the lymphocytes in the circulating blood can be considered as made up of senile cells. We are now inclined to believe, since we have seen all of the different groups of the lymphocytes in motion, that the best sign of senility in lymphocytes, judged in the supra-vital technique, is the decrease in mitochondria. Of all the types of the lymphocytes, the one in which a decrease in mitochondria can be noted is the small lymphocyte. Thus, it seems probable that if one divided the small lymphocytes into two groups, those with a moderate number and those with very few mitochondria, the latter group would represent the oldest lymphocytes in the circulating blood. We have seen a few small lymphocytes in the circulating blood without any mitochondria and we regarded them as degenerating forms. Our conclusion, from the supra-vital studies, is that the large lymphocytes are the younger types, but that the large, the intermediate, and the small forms are all mature functioning forms, except a very small part of the small types which have become senile. Of all the forms, however, it is those which are intermediate in size that are most frequently seen in active motion.

In the fixed preparations, we have made a further subdivision of the lymphocytes, and this is shown on Chart 11 F. Again, we recorded the cells in the three groups according to size, but within these three groups we divided all the lymphocytes into

those that did and those that did not have azurophilic granules in their cytoplasm. In general, on this basis, the lymphocytes can be divided into two groups, those that have a rather intensely basophilic cytoplasm with no azurophilic granules and those that have a faintly basophilic cytoplasm and contain azurophilic granules. None of the cells that have the more basophilic cytoplasm contain the azurophilic granules, but only a part of the cells with faintly basophilic cytoplasm do contain the granules. Of the small lymphocytes, all have the more basophilic cytoplasm and hence contain no azurophilic granules. Of the intermediate cells, the majority have the more basophilic cytoplasm with no granules; while a lesser number are faintly basophilic and do contain granules. The second line from the bottom on Chart 11 F shows the number of pale intermediate cells with azure granules, which is to be compared with the total of the intermediate cells on the same chart. All of the large cells have the faintly basophilic cytoplasm, and approximately half of them contain granules. The numbers with and without granules we have found about equally distributed in each count, and therefore have not separated them on the chart. We are unable to correlate the azurophilic granules with any substance that we see in the living cells. They certainly do not correlate with the vacuoles that stain with neutral red, which we regard as signs of function of a slight power of phagocytosis on the part of the cell, because these vacuoles occur in all three types of lymphocytes, though least in number in the large types of the cells. When they occur, they will be found in every cell and definitely not in the restricted numbers in which the azurophilic granules are found.

When we plot the different types of the lymphocytes, we find that the total number of the cells has a wider range of fluctuation than the leucocytes, and within the group of the lymphocytes the fluctuations between the types are even more marked. This is most striking in the contrast between the small and the intermediate forms. On Charts 10 F, 12 E and 13 B, we have shown the lymphocytes plotted in hundreds, but on the same scale of abscissae as our other curves. This method accentuates to the eye the fact of the fluctuations within the group. When all of our curves of lymphocytes are compared, the striking point is that there is a constant shifting of the small and intermediate forms, with a fairly constant reciprocal relationship between them; when the number of the small cells is large, the number of the intermediate cells is small, and the rhythm is repeated fairly regularly every half hour. In fact, it is the relationship of these two types of the lymphocytes that stamp the half hourly rhythm on the curve of the total lymphocytes.

Chart 10 F shows very well the reciprocal relation of the small and intermediate forms and in this chart the range of variation is not excessive; that is to say, the actual numbers of the small and intermediate types are approximately equal. That this is true in general will be seen by comparing the percentages of the two types on Tables 4 and 5. On

Chart 10 F the large lymphocytes run at a very low level and not infrequently are missing. Chart 12 E is a very representative one, with the alternation of the small and intermediate forms occurring fairly symmetrically. In this chart the large lymphocytes are also few in number, with occasional increases at irregular intervals.

Chart 13 B shows the same type of rhythm of the different forms but, in this instance, there are two very marked peaks of the small lymphocytes at 11:45 a.m. and at 1 o'clock, respectively, while during the afternoon hours the small lymphocytes fall almost to the base line. It will be noted on Chart 1 A that there were two very marked peaks of the lymphocytes at 11:45 a.m. and 12:45 p.m. At the time of these two increases in total lymphocytes the number of the small cells were 0 and 5 per cent respectively, while the total lymphocytes were 31 and 37 per cent respectively. In these two cases the lymphocytes were predominantly large forms at 11:45 a.m. and equally divided between large and intermediate forms at 12:45 p.m. If the term large lymphocyte is to be restricted only to the very largest of the lymphocytes that very rarely come into the blood under normal conditions, then it is possible that we should have classified these very marked showers as larger intermediate types, but our records indicate that there may be very marked showers of these larger types of lymphocytes under entirely normal conditions. We do not regard this series of studies as extensive enough to analyze this point, but we do think that they show that the point could be further analyzed from more extensive studies of the peripheral blood and even better from careful studies of the rhythm of the different types of lymphocytes which could be made from the lymph obtained from a fistula of the thoracic duct.

In general, as far as we have gone in our studies of the rhythms of the lymphocytes, we are quite certain that there is no such degeneration of lymphocytes in showers as is characteristic of the leucocytes. The leucocyte is a cell which can no longer divide and dies out in large numbers in the circulating blood. This difference between the neutrophilic leucocytes and the lymphocytes, we (Cunningham, Sabin, Doan, page 265 (13)) expressed in our chart of the different levels of the white blood-cells in which we indicated that the neutrophilic leucocyte was to be considered as having reached a further stage in maturation than either the lymphocytes or the monocytes of the circulating blood, a stage in which it was still capable of functioning and of active movement, but in which it had lost the mitochondria and possibly the centrosome, as well as the capability of dividing to produce daughter leucocytes.

In the case of the lymphocytes of the circulating blood, the degenerating cells that we can identify as having come from them practically never amount to more than one or two in a hundred at any one time and make less than 1 per cent in the average of the counts of any experiment. This is true both in the vital preparations and in fixed films. In the supravital preparations we occasionally

find a small cell without mitochondria whose cytoplasm is vacuolated and contains one or two highly refractive droplets; these we have classified as degenerating lymphocytes. Corresponding to them are occasional small cells in the fixed films with vacuolated cytoplasm that we have classified as damaged cells and consider as coming from the lymphocytes. In cases of lymphatic leukemia we have found great masses of smudges, in which it appears that the nuclei and cytoplasm degenerate together as distinct from the characteristic condition in the case of the leucocytes in which the nuclei are more resistant. In normal blood, however, it is quite certain that there is not sufficient death of lymphocytes in the circulating blood to be significant. It will be remembered that Bunting and Houston (7) showed that more lymphocytes enter the blood-stream each day than can be demonstrated in the blood on the basis of its total volume and they therefore postulated that vast numbers of them leave the blood-vessels of the alimentary tract each day. The absence of any considerable number of dying lymphocytes in the blood-stream correlates well with Bunting's theory.

It seems to us that the marked and very rapid alternation of the small and intermediate types, with an occasional shower of larger forms, suggests that different types of lymphocytes may come into the circulation rhythmically, but the question is greatly complicated by the probability that large numbers of lymphocytes leave the circulation. We are well aware that our series is far too small for us to formulate sharply the nature of the rhythms of the lymphocytes in the circulating blood, but we feel that the fact that there are no showers of dying cells can be established quite definitely. The subject must next be studied from the standpoint of the intake of the cells alone, and this can be readily done by determining the rhythms of the different forms of lymphocytes obtained by a study of the lymph of the thoracic duct. After this has been done, we can make a better analysis of the rhythms of the circulating lymphocytes. In this connection the differences in the numbers of lymphocytes in the heart's blood of the rabbit and in the peripheral blood (Chart 18) are suggestive.

We have as yet had no satisfactory demonstration as to which type of lymphocyte in the circulating blood represents the less mature and which the more mature form. As is well known, opinions differ markedly in this regard. We make a tentative suggestion from our studies that the great mass of the lymphocytes, which are the ones with rather markedly basophilic cytoplasm, are more likely to be the mature forms and this group includes most of the intermediate types and all of the small cells. The fact that it is the intermediate forms which are most commonly to be seen in motion is in agreement with this theory. We do not think that the small lymphocyte can be regarded as a senile cell, however, because it can move and also has many mitochondria. We correlate with an inability to divide, such as we find characteristic of the leucocytes, three factors in placing a cell into level 5

of our table (Cunningham, Sabin, Doan (13), page 265), namely; the entire loss of mitochondria, the loss of the centrosphere and the change in the nucleus which we call "polymorphism." The small lymphocyte is not in this level. We think that these cells are still able to divide and we are sure that almost all of them have mitochondria in their cytoplasm. If smears are made from the freshly cut surface of a lymph gland and stained with Wright's blood stain, it will appear that there is a very great predominance of cells that have less basophilic cytoplasm than the lymphocytes of the circulation; and supra-vital studies of such material show that such cells of the lymph glands, the lymphoblasts, are heavily loaded with mitochondria. On this basis, then, the large lymphocytes and a small part of the intermediate types, those that have the faintly basophilic cytoplasm, might be considered the less mature forms. Of these cells with faintly basophilic cytoplasm, many of the intermediate types and half of the large types have azurophilic granules. In general, then, it has seemed to us that the lymphocytes become progressively more basophilic as they mature. This is in sharp contrast with the findings we have published elsewhere (13) regarding the maturation of the myeloblastic series. The myeloblast first differentiates sharply from the primitive cell by becoming very basophilic; then, as it matures into the myelocyte, it loses this basophilia coincidently with the development of the specific granules.

In connection with the lymphocytes we have a very complicated question to analyze. What is the mechanism by which the total number of the lymphocytes is kept so constant, while there are such marked and frequent fluctuations between the small and the intermediate forms? How is such a rhythm to be explained with reference to the entrance into and the loss of the lymphocytes from the circulation? The presence of a valve between the thoracic duct and the veins makes it easy to postulate a rhythmical entrance of lymphocytes into the circulation by an appropriate activity of the valve. It is clear that the next step for an analysis of the rhythms of the lymphocytes is to analyze the types of the cells as they enter the blood stream, in order to determine if the rapid alternation of small and intermediate forms is characteristic of the entrance of lymphocytes into the circulation. When this factor is known, it may then be more feasible to decide what the effect of the lymphocytes leaving the circulation has on their rhythm. At present we can only conclude that the rhythm of the lymphocytes is a much more rapid one than that of the leucocytes and that the striking contrast between the two types of white blood-cells is that one type, the leucocyte, dies off in marked showers in the circulating blood, while the other type, the lymphocyte, does not.

### RHYTHM OF THE MONOCYTES

In studying the monocytes, it is first necessary to define the cell from the standpoint of both the fixed smear and the supra-vital technique.

In 1923, Sabin (34), in studies made on living blood-cells, described a large mononuclear form and also a transitional type of the monocyte. We now know this to have been a mistake, because the cell she called a large mononuclear cell in the supra-vital technique was really a myelocyte which was found in normal blood. This type of cell, shown by Sabin (Fig. 3 (34)), will be discussed under the myelocytes of our series. There is, then, but one type of cell, namely, the transitional form of Ehrlich, or, as it is now termed, the monocyte of Naegeli. We are thoroughly convinced that the double terminology of large mononuclear and transitional cells should be dropped and the term "monocyte" used.

We believe that the discrimination of monocytes from lymphocytes (for it is only with the lymphocyte that the monocyte may be confused in studies of blood) is rather more conclusive in supra-vital preparations than in fixed films, although even in the supra-vital technique there is an occasional cell which it is very difficult to classify. The great majority of the monocytes are large cells; no other cell of normal blood is so large except an unusually large lymphocyte. But there are always a few small monocytes, half the normal size, and some that are intermediate in size between these two groups. In supra-vital preparations, when the cell is rounded up and not moving, the nucleus is generally eccentric and kidney-shaped; and opposite the *Hof* of the nucleus there is a definite rosette of bodies that stain a characteristic salmon tint in neutral red. Of the bodies that stain with neutral red, making this rosette, those in the center are generally small, while those in the periphery are larger; the peripheral bodies may become quite large and under certain conditions all the bodies that stain in neutral red are large. Surrounding the rosette are numerous mitochondria. The monocytes that occur in the circulating blood may be roughly divided into two groups; the usual, apparently inactive type, and an extremely stimulated one. The latter do not occur normally, but are found in Malta fever and also in tuberculosis. One such monocyte, from a case of Malta fever, was shown by Sabin (34) on Plate XXXV, as Fig. 5. Figure 4, on the same plate shows a wholly normal cell of the same type. It is obvious that in the highly stimulated form of Fig. 5, the rosette, so characteristic of the monocyte, was still retained. We have given elsewhere (36) further descriptions and drawings of monocytes, both in more primitive forms than are found in the normal circulating blood, and also as cells which have been experimentally stimulated to great activity. In general, the monocyte is characterized by the peripheral position of phagocytized material.

In fixed preparations, we have based our studies of the monocytes on Naegeli's description. We have used Wright's blood stain throughout and, in this technique, the basophilic cytoplasm of the monocyte takes on a peculiar muddy blue color which is quite distinct from the clear blue of lymphocytes. The monocyte has a rather deeply basophilic cytoplasm when stained with eosin-azur, and

scattered throughout its cytoplasm are fine red granules, first described by Naegeli. These granules we cannot identify with anything that we see in the living cell. They are not in a rosette.

The most marked characteristic that we have made out about the monocyte, from our studies, is its remarkable power of cell-division. We have therefore classified monocytes, lymphocytes and certain myelocytes as all belonging to the level of the blood cells that retains the capacity to divide (Cunningham, Sabin, Doan (13)). In the circulating blood of our experimental animals we have repeatedly found monocytes undergoing division. The type of division which we have consistently found in the monocyte of the circulating blood is amitosis; that is to say, we find cells in which nuclear division has taken place, while the undivided centrosome remains between the two nuclei; or we find a cell in which nucleus and centrosphere have divided, but the line of cleavage for the cytoplasm is between the two centrospheres. We have therefore given the delayed division of the centrosphere as our criterion of amitosis (Sabin, Doan, Cunningham (36)). In all the series of differential counts presented in this study, in which the number of the cells runs up into the thousands, we always obtained some evidence of division of the monocytes, for we have found, in every case, a few small monocytes of half the average size which we think are the daughter cells from monocytes which have just divided. Thus, in the series of Chart 6 F, in which 5600 cells were counted, we recorded 5 which were half size. There are, of course, always more cells of intermediate size, but we have not systematically recorded them. It is these small monocytes, just after cell division, which are difficult to discriminate, both in supra-vital and in fixed films, because they are sometimes quite similar to intermediate lymphocytes. In supra-vital preparations, the intermediate lymphocytes occasionally have more of the bodies that stain in neutral red than usual and, while the lymphocyte never has a rosette, nevertheless, a lymphocyte with many stained vacuoles may be puzzling to differentiate from a small monocyte in which the rosette has become obscured. We consider that the recognition th at there are small monocytes, half the normal size, will be an aid in the discrimination between monocytes and lymphocytes in fixed films.

We are giving one chart (14 F) of the monocytes plotted on the same scale as the neutrophilic leucocytes of Charts 1 to 6, to show that, in comparison, their variations are slight, but that their rhythm is similar to that of the leucocytes. When the monocytes are plotted on a scale of hundreds instead of thousands, as is shown on Charts 15 D and 16 B, it becomes more obvious that the monocytes differ strikingly from the lymphocytes in having a slower rhythm. They are markedly rhythmical, but they have a longer interval; on Chart 16 B, the interval is almost 2 hours, but on Chart 15 D, they have practically the same hourly interval as that of the leucocytes. Very rarely the curve of the monocytes drops to the base line. There

is no consistent rise of the monocytes in the afternoon.

### RHYTHMS OF THE BASOPHILIC AND EOSINOPHILIC LEUCOCYTES

Both the basophilic and the eosinophilic leucocytes, as shown on Charts 14 F, 15 D and 16 B, run on an hourly rhythm and show rather uniform types of curves. The point of difference between the two is that the basophilic leucocytes are much more frequently absent altogether. Thus, for Chart 15 D, basophiles were absent in 16 out of 33 counts. It will be remembered that we counted only 100 cells in all but one of the supra-vital series, and it is clear that had we been able to count a greater number of cells we would certainly have found basophilic leucocytes in a greater percentage of the counts.

The supra-vital technique brings out very sharply the fact that the basophilic cell in the circulating blood is an active living type. The reaction of the basophilic granules to neutral red is irregular in shade in the human cell, in contrast to the uniform color in the basophilic cells of other animals, but all the shades of red in the basophiles incline markedly toward the acid reaction of the dye, while the red of the granules of the eosinophiles tends toward the alkaline reaction. We are sure that the basophilic cells are living types with no signs of degeneration, because in living preparations their nuclei do not stain and they have active amoeboid motion. We occasionally see a non-motile cell in which the granules are so very large that we think it must have been an eosinophilic leucocyte. We have occasionally recognized a degenerating basophile in supra-vital films, and can occasionally find a few fragile basophiles in fixed smears. It may be that both basophiles and eosinophiles have a non-motile phase, but such non-motiles are necessarily so few in number that they are difficult of analysis.

### THE OCCURRENCE OF MYELOCYTES IN THE CIRCULATING BLOOD

We have already mentioned—and we wish to make the correction clear—that the cell described by Sabin (Fig. 3, Plate XXXV (34)) as a large mononuclear form was actually a myelocyte. Of this we became sure as we studied the cells of bone-marrow (Doan, Cunningham and Sabin (14)), and the cells of leukemic blood, (Sabin, Austrian, Cunningham and Doan (35)). The cell of Fig. 3, noted above, was a myelocyte just at the stage when it was about to become a leucocyte; this cell has sometimes been called a meta-myelocyte. Its nucleus was slightly indented. Such a cell had its entire quota of neutrophilic granules, but had not yet lost all of its mitochondria, whereas the usual leucocyte of the blood in the adult has lost all mitochondria. As seen in the living preparation, the granules of such a cell show no movement at all, or if the cell is just passing into the stage of the leucocyte, there will be a slight moving of the granules, but no such streaming as accompanies amoeboid movement. It is specifically this late form of the myelocyte that we have found in normal blood.

Such late myelocytes we found in

four of our six series of supra-vital counts and in five of the series in the counts of fixed films. Only in one case out of six—the one shown in Chart 3 C—did we fail to find any myelocytes whatever. They occurred in occasional spurts, as is shown by upright lines on Chart 15 D, in which they were present twelve times in 33 counts in numbers ranging between 1 and 2 per cent. This blood had more myelocytes than any of our series, but as seen on Table 4, the final percentage from 3300 cells counted was only 0.3 per cent. We think that the presence of occasional myelocytes of this type in practically normal individuals may indicate a response of the bone-marrow to mild chronic infections, and may prove worth taking into consideration from the clinical standpoint.

## GENERAL DISCUSSION

From the preceding data we conclude that the white cells of the blood have a characteristic rhythm. We have found this rhythm to be fairly constant for the 7 to 8 hours of our experiments. It appears that the white blood-cells, as a whole, tend to run higher in the afternoon than in the morning, quite independently of whether food has been taken or not. There may be many factors concerned with this phenomenon; with regard to many of them, such as the effect of acid in the stomach, the changes in the acid-base equilibrium of the blood, the redistribution of the leucocytes correlated with vasomotor phenomena, we have no evidence at present. There is, however, one factor, that of a possible concentration of the blood due to the excretion of the kidneys, which we think that we can rule out, because such a concentration would affect all of the different types of the white blood-cells alike and our records show that this is not the case. Moreover, such a concentration should also affect the red blood-cells, but Chart 4 D indicates that the curve of the red cells does not show a corresponding rise in the afternoon.

When the different types of the cells are studied, it appears that the leucocytes show a rhythm that is most closely correlated with the rhythm of the total number of cells, and the lymphocytes show the least correlation with this total number. We have found that all of the types, except the lymphocytes, run on an approximately hourly rhythm, while the rhythm of the lymphocytes is more rapid, with a tendency to change every 15 minutes, making a half-hourly rhythm. Of the neutrophilic leucocytes alone is the afternoon rise characteristic. The neutrophilic leucocytes die out in numbers approximately 6 per cent of their total for the eight hours studied; if this rhythm is maintained for 24 hours, then about one fifth of the neutrophilic leucocytes die in the circulating blood each 24 hours. If there is any such rhythm of the blood-cells as our curves seem to indicate, then there is a much greater variation in the actual numbers of the different types of white blood-cells per cubic millimeter than is regarded as normal in the current teaching of clinical microscopy. We are showing in Tables 4 and 5 the average percentages of the different types of cells

from the differential counts made with the supra-vital technique and with the counts from fixed films. In Tables 6 and 7 we are giving our range of variation both in percentages and in actual numbers.

When the average percentages are compared (Tables 4 and 5), it appears that the neutrophilic leucocytes of the supra-vital counts run 6.32 per cent higher than in the differentials from the fixed films, but the total leucocytes, motile and non-motile combined, are only 2.35 per cent higher, while on the other hand the lymphocytes run practically the same amount, namely 3.58 per cent, lower. We have tried to analyze this difference between leucocytes and lymphocytes in the two techniques. There is the possibility that we miss some leucocytes in the fixed films through their becoming damaged in the technique; we think, however, that we cover this point by recording all fragile leucocytes, all pale cells and all free nuclei, with or without granules in the neighborhood. It has been suggested that there is a possible factor of error in counting a few leucocytes twice in the supra-vital differentials, because those that are near the edge of the field might wander into the field as one moves along the row, but it is obvious that this would not give a consistent increase, for the cells would as frequently move out of the field as into it. Furthermore, we do not think that the actual rate of movement of the leucocytes is sufficiently rapid to make this factor very important. On the other hand, we may miss a few of the lymphocytes in our supra-vital counts. The lymphocytes are the least conspicuous cells in the supra-vital preparation and it is possible that with the speed which is necessary in order to do the experiment at all, we may consistently miss a few lymphocytes in our counts. Moreover, as we stated in the beginning, the films for these experiments were made consistently thick enough to have some overriding of the red cells. If we have missed any lymphocytes in the supra-vital counts it was not consistently the small forms, for we have made very careful comparisons between the two methods on this point. When the other types of cells on Tables 4 and 5 are compared, it will be seen that the differences between the two techniques are likewise consistent for them. Thus, the basophiles and the eosinophiles run higher in the supra-vital counts than in the fixed films, and a comparison of the two tables shows that this difference is entirely consistent for each experiment. We feel confident that the supra-vital technique is more sharply differential for these two types of granules than is the stained preparation. This discrepancy is accounted for by those cells in which the staining in eosin-azur is not sharply differential for the basophilic or eosinophilic granules, and such doubtful cells are commonly classified with the neutrophilic cells. There is no such doubtful group with neutral red, which is a chemical indicator, and this accounts for the higher percentage of these two types of cells obtained by the supra-vital technique, In the case of the monocytes the conditions are reversed, for it will be seen that their percentages are slightly lower in the supra-vital technique. In general, we do

not think that every single cell can be discriminated in the supra-vital technique but we are inclined to consider that the discrimination is actually a little sharper with this technique than with the staining of fixed films. In spite of these differences in the comparison of the two techniques, our records in both are, of course, close to the accepted normal percentages (see Tables 4 and 5).

When we come to show our range of variation, however, as seen in Tables 6 and 7, we come into conflict with the limits that are more commonly accepted as normal. Thus, Naegeli (28) gives the normal limits of variation of the neutrophilic leucocytes as from 65 to 70 per cent, with the actual numbers varying from 4500 to 5000 cells. These figures are the limits most generally accepted, nevertheless, there have not been lacking evidences in the literature that such limits are too narrow. Thus Galambos (16) gave 3500 to 12,500 as the normal limits of the total number of white blood-cells in human blood with the range in an individual at different periods of the day as almost 100 per cent. It is with his findings that our records agree; thus, it will be seen in Table 1 that our extreme limits between the lowest counts and the highest are from 4200 to 13,680, but we do not think that these limits are the most significant data because our table shows that these extreme limits do not obtain in any one individual, but rather that for a given individual the actual numbers are in the ratio of 1 to 2. Thus for Chart A 1 (see Table 1) the range is high, running from 7200 to 13,680, while Chart 6 F (see Table 1) the range is a low one, being from 4200 to 8700. We would, therefore, state our results as indicating that for each individual the highest number of the white blood-cells in any day tends to be twice the lowest number. For the neutrophilic leucocytes, our average range of percentages is from 39 to 76 per cent, the actual average numbers varying from 2830 to 7537. In this wide range of variation of the leucocytes it is clear that the lower level is due to our differentiating the neutrophilic leucocytes into motile and non-motile types in the differential counts; thus, if at certain times half of the leucocytes are found in the non-motile phase, it is obvious that the lowest limit must be approximately half of the usual percentage of these types. This is well shown on Chart 17 B. If we take the sum of the motile and of the non-motile leucocytes, the range of variation in percentage becomes 56 to 78 per cent.

We have not found that the lymphocytes die out in the circulation in any great numbers; nevertheless, we have noted with them also a marked range in variation. For the lymphocytes Naegeli has given 20 to 25 per cent as the limits, with 1500 to 2000 as the actual numbers of the cells. Again, there are evidences in the literature in the work of Galambos, Torday, and Schilling that these limits are too narrow, Galambos giving 50 per cent as the upper limit, Torday giving the range as from 13 to 40 per cent and Schilling reporting that an upper limit of 30 to 40 per cent is not uncommon. Our average limits are from 10 to 31 per cent,

the actual numbers being 756–2367. In the two experiments shown in Charts 1 A, and 4 D, there were two counts with a low limit of 6 and 8 respectively. These limits we regard as too low; they occurred only twice in the two experiments and all of the other low counts in these two experiments ran around 12 per cent. In our judgment, they were accidental low counts which would have been changed had we counted three or four hundred cells instead of the one hundred. It is generally conceded that there is a range of extreme variation which is eliminated by counting a sufficient number of cells, but as explained at the start, our experiments could not have been done at all with our limited group if we had counted more cells for each interval. We believe that a lower limit of 12 is more accurate than the limit of 10; and that the average upper limit is about 36. Thus, we think that the range of variation of the lymphocytes is about 1 to 3, that is to say, that at the highest points there are about three times as many lymphocytes in a drop of blood as at the lowest points. These limits are to be compared with those for the leucocytes, which seem to us to be about 1 to 2. We find that the range in percentage for the basophiles is from 0 to 5 per cent, while the range both for eosinophiles and for monocytes is about from 1 to 10 per cent. Our experience has been so consistent in regard to this much greater range of variation in the different types of the blood-cells than is the more generally accepted opinion, that we are quite convinced that our limits of variation come much nearer being representative of what is actually taking place in the circulation than are the accepted figures. We think that our studies show that there is no constant level of the total white count for a day, and no constant level for each type of cell, but that there is a rhythm of the white blood-cells just as there is a rhythm of the temperature. Such a rhythm as our curves show brings out the meaning of the studies in the literature which have indicated that the variations in the number of the cells are much greater than the accepted values. Our curves show that each type of the white blood-cells has its own characteristic rhythm, of which the general curve is the compound. If this be true, it is more than ever remarkable that the number of the white blood-cells in a drop of blood remains so nearly constant, from hour to hour and from day to day.

Our records show that there is a rise in the white blood-cells, due to an increase in the neutrophilic leucocytes, in the afternoon, regardless of whether lunch has been taken or not. Among all of the extensive studies of digestive leucocytoses in the literature, Japha (21) and Türk (42) are the only ones whose results agree with ours. Their work also indicates that the subject of a digestive leucocytosis must be studied in connection with a daily variation, which we have shown is a characteristic rhythm.

We began these studies with the concept that if we could find evidence concerning the amount of the cell-death we might obtain some evidence concerning the replacement of these cells in the circulating blood. We have shown that there is almost

constantly an immediate as well as a delayed increase in the motile cells following showers of dying cells. However, we do not think that we are justified in drawing the conclusion that these increases represent an influx of new cells from bone-marrow until we have analyzed how much the factor of the relative shifting of the proportions of the different cells, already actually within the circulation, affects the rhythms we have disclosed. The nature of these rhythms, however, we believe offers a basis for further study of this problem. We need to know to what extent and in what way these rhythms represent the advent of new cells in order to study the stimuli that call these cells into the circulation.

It seems to us that these studies on the rhythms of the white blood-cells open up a large number of problems to be investigated. Among these, the most obvious are; an attempt to correlate the rhythms of the blood-cells in different parts of the vascular system, with especial reference to the peripheral capillaries and the deeper vessels; the study of the rhythms of the lymphocytes from the thoracic duct; the study of the various types of cells occurring in the splenic veins and the veins of the bone-marrow; and finally, an analysis of the rhythms of these cells as found in the leucopenias and leucocytoses of disease.

SUMMARY

1. There is a characteristic rhythm of the total white blood-cells with an interval of approximately an hour's duration.

2. The total number of the white blood-cells varies in the proportion of 1 to 2.

3. The total number of the white blood-cells is increased in the afternoon regardless of whether food has been taken or not, and this entire increase is the result of an increase in the number of the neutrophilic leucocytes.

4. The neutrophilic leucocytes die out in showers, often of considerable proportions. These dead cells are promptly replaced, though whether from some reservoir or directly from the bone-marrow, we have not yet determined.

5. The lymphocytes have a shorter rhythm than the leucocytes, but their total number varies in the proportion of 1 to 3, in contrast to the variation of 1 to 2 for the leucocytes.

6. A small proportion of the monocytes are normally undergoing division in the circulating blood.

7. In five out of six apparently normal individuals, small percentages of myelocytes were found in the circulating blood.

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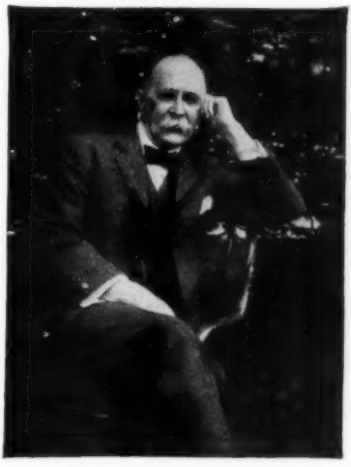
*The Life of Sir William Osler.* By Harvey Cushing. Vol. I, 685 pages. Vol. II, 728 pages. 1925. 8°. The Clarendon Press, Oxford. $12.50.

# Book Reviews

*Sir William Osler.* By HARVEY CUSHING. Vol. I, 685 pp. Vol. II, 728 pp. $12.50. *(Oxford at the Clarendon Press.)*

Two special audiences, I think, must have been present in Dr. Harvey Cushing's mind, while he was shaping the plan and filling in the details of the life of his friend and teacher. One of these is indicated in his words: "Those who knew him best will appreciate the difficulty of compiling these present records, which are *mémoires pour servir* . . . . Here are merely the outlines for the final portrait, to be painted out when the colors, lights and shadows come in time to be added. . . . ." In writing for the future, Dr. Cushing had his most difficult problems. Feeling that he held at the point of his pen posterity's judgment of one of the great human figures of our time, a man so loved and venerated by all who knew him that any other feelings than these seemed almost an impertinence, he yet had to present him as far as possible without personal prejudice, choosing impartially from an enormous material that which threw a light on all sides of his character. Upon how completely he has succeeded in this object, how masterfully he has welded his mass of detail into a sympathetic but faithful picture of William Osler, the man and the beloved physician, I shall leave others better qualified in literary criticism than myself, to decide. It is that other audience, more special than this, that I would here speak of, indeed, speak *to*. It was not without a very definite purpose that these volumes were dedicated to: "Medical Students, in the hope that something of Osler's spirit may be conveyed to those of a generation that has not known him; and particularly those in America, lest it be forgotten who it was that made it possible for them to work at the bedside in the wards." More than to any other group of men, it was to medical students that Osler's life was lived, and so appropriately it was to them, above all, that this biography was written: bearing always in mind that even in later life, the best physician is he that keeps a portion of the student spirit alive within him, never too old to learn or share new enthusiasms in research or reform, and who is still an ardent hero-worshiper at the shrine of the great masters of the profession.

"Education" may be understood in two ways. There is the narrower sense, the education of the reasoning faculty, for



which we go to school to fill our minds with a large number of facts, and, if we are lucky, to obtain a certain power of observation and of making deductions therefrom. But there is also a larger education, not of the mind alone, but of the man, an education which begins with birth (perhaps before) and ends only with life itself. It consists in the reaction of the individual to everything he meets, everything, that is, which makes a significant mark on his attention. In Samuel Butler's trenchant metaphor, a man is a cross with everything in his environment that influences him either for good or evil. Now, the most important

influences in this larger education are the human associations of youth; the most important of all to the student are his teachers. Their inspiration and example will bear far riper and more enduring fruit than any amount of book-learning which goes by the name of education today. "In the teacher," wrote Osler, "I have always valued the message of the life, above the message of the pen." He recognized this influence in his own life, and with characteristic generosity dedicated not alone his text-book, but a large part of all his power and success in medicine to the three teachers, W. A. Johnson, James Bovell and R. Palmer Howard. But though the immediate personal encounter with the beloved teacher must be the most powerful of all influences on the developing spirit, Osler was not willing that anyone, more than himself, should forego another less direct but scarcely less fruitful encounter, with the great teachers of the past. He would have every medical student cross himself with Plato and Sir Thomas Browne, with Rabelais and Ambroise Paré, with Harvey and Sydenham, with Lister and Pasteur. These and many others were constantly the themes of his addresses to students, both in this country and in England, and in all of them it is not alone the accomplished work, but the lesson of the man's life on which the attention of speaker and audience is focussed. To such names as these, that of Osler himself must now be added. To hundreds of men and women still living, the memory of his friendship or even of a chance meeting will be ever one of their most treasured possessions; to many, he must have been a deciding influence in their lives. But the present generation of students must find him, if they would find him at all, in the legends which cling about his name wherever he passed, in his own writings, and above all in these volumes. If there was ever a doubt, that the wonderful lesson and example of Osler's life might have been lost to generations beyond his own, this doubt has now been happily resolved. In future, the medical student who has failed to cross himself with William Osler will have only himself to blame.

Of the many lessons which Osler used to impress into his students, and of which his own life was the best example, I would like to mention four, not in the least that they include the whole of his message, but which I think it may help to bear in mind while following him through two long and intricate volumes. The first is his insistence on *work* as the "master word in medicine." Nothing worthy can be accomplished without hard unremitting labour. Himself always the busiest of men, both in and out of work hours, he never put off (for a moment) anything which, in his opinion, needed to be done. His favorite quotation was: "The flighty purpose never is o'ertook unless the deed go with it," and scarcely a day passed when he had not occasion to use it either for himself or another. In his "Way of Life" he warns the student against dreaming of the future or brooding over the past. Each day contains enough to absorb the whole of his teleological faculties. The second lesson is Aequanimitas, a spirit of epicurean moderation in all things, success and failure, joy and sorrow. In the realm of action the same sense for realities led him to shun extremes as liable to defeat the desired end; "Le mieux est l'enemi du bien." Thus, he always took his stand openly with any group, professional, local or national, to which he belonged, thereby making it possible for him to wield a powerful modifying influence within that group. The third is that breadth of sympathy which can only come through breadth of knowledge: knowledge of men, of books, of peoples. Narrow-mindedness born of misunderstanding is the great hindrance to progress and happiness on earth. Lastly, his belief in Faith as the strongest and most beneficent influence in human affairs. "Faith is the antiseptic of the soul," said Walt Whitman: Osler would have added, "and often of the body also." The penetrating faith of men like Plato and Sir Thomas Browne so caught his imagination and sympathy that he would rather share their beliefs than listen to the cold formalities of scientific or historical criticism. His own creed was the inherent goodness of human nature. He preferred to believe in the gold of men and women even when they were proved to have feet of clay. Another of his favorite couplets from Shakespeare's sonnets, which I quote from memory was—"If my true love swears

she is made up of truth, I will believe her though I know she lies." His was far from being an intellectual Religion of Humanity in the Comtian sense. It was instinctive, unreasoned, to many no doubt unreasonable, but having in this very characteristic the surest sign of a religious faith.

R. E.

*Heredity in Nervous and Mental Diseases. An Investigation by the Association for Research in Nervous and Mental Diseases.* 332 pp. $3.75. (*Paul B. Hoeber, New York.*)

This volume consists of a collection of papers read before the Association for Research in Nervous and Mental Diseases. As the papers together represent an attempt to cover most of the field of inheritance in the types of disease mentioned, they are for the most part compilations from the literature; but a few describe original work. The most striking communication is Stockard's, who records the surprising effect of alcoholism in guinea-pigs on their progeny. The result was the production in the fourth generation of a sturdier race. He gives scarcely enough weight in his paper to the possible influence of the alcoholic uterine environment on the growing embryo (an influence, the strength of which is suggested by the fact that the prenatal mortality is doubled in the immediate offspring of alcoholic parents, as compared with the normal), but makes amends for this in his "answers to questions" printed as an appendix. Stockard is careful to point out that his data do not illustrate heredity in the Mendelian sense. Barrett gives an excellent summary of the literature on heredity in the psychoses. He accepts rather uncritically Kretschmer's schizoidy and syntony, treats them as unit characters and flirts with Hofmann's view of schizophrenia as the result of two inherited components—a peculiar mentality (schizoidy) and a characteristic type of deterioration (the schizophrenic process *Anlage*). From this the questions will inevitably arise: How do schizophrenics get well? and, What is the rôle of the environment? Orton gives a succinct account of the pathological changes in hereditary and familial types of nervous and some mental disorders. It seemed hardly necessary to poison rabbits with oxalic acid to prove that nervous death may occur without structural change in the nerve-cells, this being the basic assumption of neurohistopathology. Occam's razor seems to disfigure the fair face of Orton's hypothesis that "catagenesis" (degenerative change) and precocious senility together account for the heredodegenerations. Jelliffe's erudite summary emphasizes the greater incidence of disease on the phyletically younger portions of the nervous system. His psychoanalytic bias leads to a curious insistence on what he calls "incest" as a factor in the heredodegenerations—because all mankind are said to be related as nearly as thirtieth cousins. Jelliffe seems to embrace Josephy's findings of organic change in the cerebral cortex in dementia praecox, and he would courageously refer the motor phenomena of this disorder to organic disturbance of the corpus striatum. Evidently Jelliffe and Barrett hold precisely opposite views as to the value of the evidence for the hereditary basis of essential epilepsy, the former rejecting most of the work previously done on this problem.

Lewis describes a miscellany of extraneural pathological processes in various familial and hereditary diseases. His findings, including the endocrine changes in epilepsy and mental defect, are interesting, but the ages of the subjects autopsied, their medical history, and the causes of death, are not mentioned, and no control material is dealt with. There appears in several places Lewis's curious concept of the concomitance of organic compensatory hypertrophy and psychic compensation—a new and bizarre kind of psychophysical parallelism which we suspect will prove to have little more than a linguistic basis.

Division of labor among an editorial board has presumably been responsible for the absence of an index, and for the want of an adequate table of contents, and of a glossary of terms, the last of which Dr. Dana's paper only partially supplies. There are various misprints—e.g. "principal" for "principle" (p. 63), "than" instead of "from" (p. 273), "no" for "an" (p. 112), and so on. Dr. Kinnear Wilson will, we hope, be pleased to find that he has been knighted on page 122.

R. D. G.

*The Gold-Headed Cane.* By WILLIAM MACMICHAEL, M.D. With an Introduction by Sir William Osler, Bart, M.D., F.R.S. And a Preface by Francis R. Packard, M.D. 8° 285 pp. 21 illust. Cloth, $2.75. (*New York, Paul B. Hoeber*, 1925.)

Older medical readers have long been familiar with this delightful book, for early in his teaching, both in Philadelphia and in Baltimore, Dr. Osler introduced it to his students and to other American readers. In those days only the earlier editions were to be had—those published in England prior to 1884 and only to be found in second-hand bookshops.

In 1915 Hoeber in New York issued the first American edition with Dr. Osler's introduction, covering a brief review of the text, and Dr. Packard's preface containing a short account of Dr. Macmichael, the author, and his bibliography. The book was reprinted in 1919, and again in December 1924. The last reprint, which is now before us, appears with the date 1925 on its title page. Too much credit cannot be given to Mr. Hoeber for placing before Americans successive issues of this unique book; unique because in its conception and consistent manner of presenting the lives of five of England's distinguished doctors there is nothing similar in literature.

The Gold-Headed Cane, preserved in the Library of the Royal College of Physicians of London with the utmost care, is made to tell of its experiences as it went about with its five successive owners, beginning with the great Dr. Radcliffe in 1689 and passing on to Mead, Askew, Pitcairn and Baillie, until, in 1825, after having "been closely connected with medicine for a century and a half" it "was deposited in a corner closet of the Library" there to remain undisturbed except that "having become to certain degree an object of curiosity, my seclusion has occasionally been broken in upon by a temporary exhibition to a visitor". Happily for the cane, it has long since been removed from the dark closet where it was originally deposited and is now to be found in a fine silk-lined glass case whence it can observe the numerous visitors to the Library and occasionally, as happened so pleasurably to the writer of this review, it may come forth and be placed again in the sympathetic and reverent hand of an admirer of its history. As was said of this book by a writer in the "Saturday Review of Literature" May 2, 1925: "Its appeal is perennial" and it "merits a favored station on the shelves of every medical library and, better still, it should find lodgment among the winnowed few bedside volumes of every physician" and I may add of every medical student.

H. B. J.

*The Internal Secretions of the Sex Glands.* By ALEXANDER LIPSCHÜTZ. 513 pp. $6.00. (*The Williams & Wilkins Co., Baltimore, 1924.*)

This is the author's English translation of a new and augmented edition of *Die Pubertätsdrüse und ihre Wirkungen* (Berne, 1919). The book is far from being a mere vehicle for the publication of the author's experimental work. It is one of the finest recent contributions to the now very prolific field of endocrinology that the reviewer has seen. The excellent plan of the book, the extensive bibliographies, the generous number of photographs, and the fluency of the translation, all contribute to its value. The approach is primarily biological and physiological—human sex pathology gets only secondary attention. The author frankly admits that his clinical experience has been very limited.

The conceptions for which the Dorpat school is best known—the existence of an asexual equipotential embryonic soma, and the reversion of the adult castrate to the neutral type—are extensively dealt with. The discussion of the various types of hermaphroditism for which the author prefers the term "intersexuality" is particularly good. The chapter on glandular extracts is much less complete. Much of the more recent American work on the ovary, such as that of Corner, Evans, Alan and Doisy, has evidently not been seen by Professor Lipschütz. The author has worked with Steinach and has a first hand acquaintance with the very important work that has come from his laboratory. He accepts almost in its entirety the work of Steinach and Voronoff on rejuvenation.

M. S. G.